University of São Paulo
"Luiz de Queiroz" College of Agriculture

Tambaqui (*Colossoma macropomum*) fish burger: optimization process and evaluation of oxidative stability

Leandro da Silva Presenza

Dissertation presented to obtain the degree of Master in Science. Area: Food Science & Technology

Piracicaba
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BIOGRAPHICAL SKETCH

Leandro da Silva Presenza, born in Vitória, Espírito Santo, son of Carlos and Analdina, was raised in Itaipava, Espírito Santo. He graduated in Fisheries Engineering and subsequently completed a postgraduate degree in Applied Policy Management at the Federal Institute of Science and Technology of Espírito Santo (IFES). He worked for three years as a researcher for the Marine and Coastal Protected Areas Project (GEF) of the Chico Mendes Institute of Biodiversity Conservation (ICMBio). During this period, he discovered the applications of science in the world of food and then moved to Piracicaba, São Paulo, where he enrolled in the master's program at the Luiz de Queiroz College of Agriculture of the University of São Paulo to work in the Department of Agri-food Industry, Food and Nutrition, in the laboratory of Dra. Thais Vieira. During her studies, Leandro published scientific papers and was awarded in the Food Systems Innovation Challenge (WUR & A5 Alliance).

CONTENTS

RESUMO

Fish burger de tambaqui (*Colossoma macropomum*): processo de otimização e avaliação da estabilidade oxidativa

O pescado é uma das principais fontes de proteína à dieta humana, e vem apresentando índices produtivos cada vez maiores nas últimas décadas, pincipalmente pelo interesse dos consumidores pela ingestão dos macros e micronutrientes pelos efeitos positivos sobre os sistemas cardiovascular, cognitivo e imunológico, além de evidenciar-se como alternativa para segurança alimentar das próximas gerações. Contudo, estudos apontam a necessidade de medidas para impulsionar a produção e consumo de espécies nativas com objetivo de atingir metas sobre o consumo de pescado. Nesse contexto, o tambaqui (*Colossoma macropomum*) se destaca sendo a espécie nativa com maior volume produtivo, e estratégias que visam a inserção de produtos dessa espécie no varejo nacional podem contribuir para o desenvolvimento de sistemas mais sustentáveis com o consumo de produtos locais. Neste estudo, um modelo de otimização reaplicável foi utilizado para desenvolvimento de uma formulação de fish burger à base CMS de tambaqui, com foco na redução de custo e melhorias das propriedades físico-químicas e sensoriais. A adição da mistura de farinhas apresentou efeito sinérgico para a redução de custos, melhoria das qualidades texturais, sensoriais e o rendimento de cozimento foi 12% maior em relação a formulação sem farinha ($p < 0.05$). Considerando que o pescado é um produto altamente susceptível a deterioração, produtos convencionais à base de pescado tendem a ter uma vida de prateleira curta, nesse sentido a segunda etapa do estudo foi realizada para estabelecer um produto *clean label*. A adição de compostos bioativos vem sendo investigado como potencial agente para a extensão de vida de prateleira de produtos do pescado. Extratos obtidos de subprodutos do processamento de abacate (*Persea americana* Mill.) foram adicionados ao fish burger, e as propriedades bioquímicas e físico-químicas foram avaliadas durante armazenamento a 4 °C / 14 dias e a -18 °C / 90 dias. A adição dos extratos indicou resultados significativos para a extensão de vida de prateleira do fish burger, apesar do tratamento com eritorbato de sódio ter sido mais eficiente para inibição de produtos da oxidação lipídica (TBARS), os extratos naturais apresentaram redução no desenvolvimento de produtos da peroxidação e na formação de bases voláteis nitrogenadas (NBVT), influenciando a manutenção do frescor, perfil lipídico e a composição centesimal dos fish burgers em ambos testes de armazenamento ($p < 0.05$). De forma geral, os resultados indicaram que a utilização de subprodutos da indústria de alimentos alinhados a modelos estatísticos aplicados pode favorecer o desenvolvimento de novos produtos com aspectos de sustentabilidade e saudabilidade.

Palavras-chave: Abacate, Análise sensorial, Otimização, Oxidação lipídica, Pescado.

ABSTRACT

Tambaqui (*Colossoma macropomum*) fish burger: optimization process and evaluation of oxidative stability

Fish are one of the main sources of protein in the human diet, and it has shown production rates in recent decades, mainly due to consumer interest in the intake of micro- and macronutrients that have positive effects on the cardiovascular, cognitive and immune systems. In addition, fish stand out as a food security alternative for the next generations based on more sustainable production systems. However, studies point to the need for measures to boost the production and consumption of native species to achieve fish consumption targets. In this context, tambaqui (*Colossoma macropomum*) stands out as the Brazilian native species with the highest volume of production, and despite regionalized marketing, strategies aimed at inserting products of this species in retail can contribute to the development of more sustainable systems with the consumption of local products. In this study, a replicable optimization model was used as a strategy to develop a fish burger formulation based on mechanically separated meat (MSM) from tambaqui, focusing on cost reduction and improvements in physicochemical and sensory properties. The addition of the flour mixture showed a synergistic and significant effect to reduce costs and improve textural and sensory qualities, and the cooking yield was 12% higher than that of the formulation without flour ($p < 0.05$). Considering that fish are highly susceptible to deterioration, conventional fish-based products tend to have a short shelf life when no preservative agent is applied; in this sense, the second stage of the study was carried out to establish a clean label product. The addition of plant-based bioactive compounds has been investigated as a potential agent to extend the shelf life of fish products. Extracts obtained from byproducts of avocado (*Persea americana* Mill.) processing were added to the optimized fish burger formulation, and the biochemical and physicochemical properties were evaluated during storage at 4 °C for 14 days and at -18 °C for 90 days. The addition of extracts significantly prolonged the shelf life of the fish burgers. Although the treatment with sodium erythorbate was more efficient for the inhibition of lipid oxidation products (TBARS), the natural extracts showed a reduction in the development of peroxidation products and the formation of volatile nitrogenous bases (TVB-N), influencing the maintenance of freshness, lipid profile and proximate composition of fish burgers in both storage treatments ($p < 0.05$). In general, the results indicated that the use of byproducts from the food industry in line with applied statistical models can favor the development of new products with aspects of sustainability and healthiness.

Keywords: Avocado, Sensory analysis, Optimization, Lipid oxidation, Fish products

1. INTRODUCTION: OVERVIEW AND OBJECTIVES

Fish products describe foods of animal origin from aquatic environments comprising groups of mollusks, echinoderms, crustaceans, reptiles, and amphibians, with emphasis on marine fish or freshwater fish, which may come from extractive fisheries or aquaculture systems. In terms of production, aquaculture has stood out for its increasing production in recent years and is indicated as a viable system for the continuous and sustainable supply of animal protein to future populations (Naylor et al. 2021). According to the Food and Agriculture Organization of the United Nations in 2018, approximately 178.5 million tons of fish were produced worldwide (excluding cetaceans, reptiles, algae, and aquatic plants); in a decade, aquaculture grew by approximately 37.5%, while extractive fisheries production due to overfishing of natural stocks and greater investment in sustainable systems remained stable, with the growth of approximately 7.3% (FAO, 2020).

In Brazil, national fish farming has been growing and reached a production record in 2021 of 841.005 tons; however, even with the national potential in biological terms, especially regarding fish biodiversity, 63.5% of this production is represented by the exotic species Nile tilapia (*Oreochromis niloticus*), while the production of native species showed a result of approximately 31% (PeixeBR, 2022). Another important point in this panorama is the environmental risks related to the production of exotic species, mainly due to the lack of management found in some productions (Gama, 2008). In addition, the study by Hilsdorf et al. (2021) highlights the feasibility and importance of expanding the production of native species, indicating that these species can become commodities in the global fish market.

In addition to its cultural and productive/commercial importance, fish have intrinsic characteristics that highlight it as a key food in filling nutritional gaps, since numerous groups of fish are rich in omega-3 long-chain polyunsaturated fatty acids (LCn-3PUFAs) (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), essential amino acids, fat-soluble vitamins and minerals (Golden et al., 2021), and there is a consensus on the importance of including fish in the diet (Ruxton, 2011; USHHS/USDA, 2015).

In recent decades, the approach to fish consumption has become increasingly essential as a mechanism to support the reduction of hunger and the transition to a

sustainable food system (Nature, 2021), mainly because several authors present significant results on the performance of diets rich in fish with food security potential and in reducing the risk of the emergence of chronic noncommunicable diseases (NCDs), with positive effects on the cardiovascular, anti-inflammatory and cognitive systems (Bonaccio et al., 2017; Mohanty et al., 2019; Krittanawong et al., 2021).
Despite this productive context and dietary benefits, indices on fish consumption in Brazil are not evidenced due to factors such as lack of coordination on the management of fisheries resources at the national scale, lack of data on extractive fishing, and traditional sales. This scenario is unfavorable to establishing indicators; despite this, some studies present values referring to per capita consumption, but the data presented are not always potentially significant to represent a national average.
According to the World Health Organization, it is recommended to eat 1 to 2 servings of fish per week based on lipid composition, as nutritional performance responses will depend partly on regular consumption and the species consumed, taking into account a balance between the origin of the fish and possible environmental impacts (WHO, 2003). Therefore, there is no recommendation on the average value of fish consumption, and it is necessary to establish a parameter between which species are being consumed based on the nutritional composition and aspects of sustainability. Even so, there is a consensus in the scientific community that fish consumption at the national level is below what is necessary for the Brazilian population to benefit from positive effects on fish intake (Sartori & Amancio, 2012).
Some factors corroborate the low consumption of fish, such as food culture. The study by Cyrino et al. (2020) indicates that fish undergo a series of myths and untrue information, especially aquaculture products. The study by Pedroza Filho et al. (2020) found great consumer acceptance of Nile tilapia (*Oreochromis niloticus*) but at the same time rejection of fish from aquaculture; however, it is necessary to consider that the supply of Nile tilapia in Brazil is exclusive to aquaculture. Another factor is related to the commercialization of fish, which still maintains traditional characteristics. Consumers look for *in natura* fish in fishmongers, fairs, or even directly with the fisherman, relating the quality of the fish to storage and not necessarily to the conservation of important properties of freshness, with food

poisoning related to the consumption of fish being common (Santos, 2010; Silva Junior, Barbosa & Monteiro, 2016; Oliveira et al., 2019).

In this context, there is a need to define strategies for cultural change on fish consumption, such as the development of new products that can provide, in addition to improvements in sensory characteristics, the safety of fish products.

In turn, fish processing can provide a range of food products that meet consumers' desires, adding to the fish new characteristics and attributes such as appearance, texture, odor, color, and flavor, in addition to providing better palatability, increasing shelf life, and commercial value (Bombardelli, Syperreck & Sanches, 2005; Hall, 2010). Processing techniques in line with studies on physicochemical properties and optimization models focusing on byproducts and functional ingredients can provide fish products with promising results in terms of sensory quality, cost reduction, and full use of the raw material as a strategy for the reduction of agri-food industry waste, such as the use of Mechanically Separated Meat (MSM) (Oliveira et al., 2020; Maciel et al., 2021).

Previous studies have shown acceptability in tests with conventional fish-based products, which can mainly serve new consumers looking for foods that are easy to prepare and appeal to health (Di Monaco et al., 2009; Branciari et al., 2017; Devitiis et al., 2018). In addition, the consumption of fish species is less susceptible to social barriers and restrictive and religious diets compared to other proteins of animal origin (Tamilmozhi et al., 2013).

Aspects of healthiness and sustainability can be further explored by the food industry, such as the focus on native species such as tambaqui (*Colossoma macropomum*) (Figure 1), originating from the Amazon River basin, currently the most commercialized and widely spread native species in Brazilian fish farming, present high versatility, and acceptance of different types of food products (Embrapa, 2018; Anjos et al., 2021). The use of natural antioxidant agents can give fish-based products longer shelf life, resulting from the increase in the oxidative stability of products with a high content of unsaturated fatty acids (Speranza et al., 2009; Baptista, Horita & Sant'Ana, 2020; Shabani et al., 2021).

Figure 1: Band of tambaqui (*Colossoma macropomum*), cut most used for commercialization of the species in the Brazilian market.

Therefore, the development of conventional products based on fish byproducts under the influence of natural antioxidants in line with process optimization can contribute to increasingly sustainable aquaculture production, which can increase the economic return of the processing industries, mitigate environmental problems, contribute to the increase in fish consumption, and offer an alternative that serves consumers looking for convenience products and a clean label.

The objectives of the dissertation were as follows:

General objective: To develop and evaluate the stability of tambaqui (*Colossoma macropomum*) fish burgers with the application of avocado (Persea americana Mill.) byproduct extract as an active ingredient.

Specific objectives:

I. Optimizing the formulation of a fish burger based on mechanically separated meat (MSM) of tambaqui (*Colossoma macropomum*) with the addition of functional ingredients.
II. To evaluate the effect of independent variables (MSM; oatmeal; cassava starch) using a simplex design on the properties of yield, cooking, texture, and physicochemical composition;
III. To estimate the shelf life of the tambaqui (*Colossoma macropomum*) fish burger formulation developed with the addition of avocado (*Persea americana* Mill.) extract through biochemical, physicochemical, and microbiological analyses.

This dissertation was developed from the compilation of 1 review article and 2 original scientific articles. These were satisfactory to achieve the proposed objectives.

I. Systematic literature review based on a bibliometric survey on the use of plant-based bioactive compounds and their application in fish products, focusing on the investigation of methodological trends.
II. The original manuscript made it possible to evaluate the optimization of a fish burger formulation based on mechanically separated meat (MSM) from tambaqui (*Colossoma macropomum*) in combination with functional ingredients, indicating improvements in technological and sensorial qualities and the reduction of the cost of the formulation.
III. The original manuscript was satisfactory for estimating the effects of adding an ethanolic extract of avocado (*Persea americana* Mill.) byproducts as an active ingredient to extend the shelf life of fish burgers.

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2. TRENDS IN SUSTAINABLE FOOD CONSERVATION: CAN PLANT COMPOUNDS REDUCE LIPID OXIDATION AND EXTEND THE SHELF LIFE OF FISH PRODUCTS?

Chapter submitted to refereed international journal
Presenza, L., Teixeira, B. F., Galvão, J. A., Vieira, T. M. F. S. (2022). Trends in sustainable food conservation: Can plant compounds reduce lipid oxidation and extend the shelf life of fish products?

Abstract

New approaches to food preservation by reducing the use of synthetic compounds have highlighted active plant compounds, drawing the attention of several researchers around the world, mainly to investigate the application of these compounds in food products more susceptible to deterioration, such as seafood and freshwater fish. The objective of this article was to carry out a systematic review and bibliometric analysis to investigate trends and patterns in the literature for the topic addressed and quantitative data. The review also presents a set of relevant data on procurement, application, and trends in the investigation of bioactive compounds on shelf life extension in fish products. The review allowed us to assess that the application of active plant compounds in fish products has been more evidenced in studies from 2015. The analysis also revealed that although some species of plants and fish are recurrent in the studies, the number of coauthorships is low, demonstrating a lack of affinity between the authors of the topic. The review showed that different methods of extraction and application of plant bioactive compounds demonstrated significant effects in reducing lipid oxidation and prolonging the shelf life of fish products. However, more detailed investigations of the composition of active compounds can corroborate more promising data regarding commercial viability.

Keywords: Antimicrobial, lipid oxidation, essential oils, phenolic compounds, plant compounds.

2.1. Introduction

Seafood and freshwater fish are among the main sources of animal protein worldwide (Golden et al., 2021); in 2018, the world production of fish was 178.5 million tons (FAO, 2020). Consumable fish can be obtained through fishery catch or aquaculture systems, with emphasis on the latter, described by many authors as a sustainable mechanism for the continuous supply of animal protein to the human population (Gephart et al., 2021). In addition, fish consumption is related to health aspects, as it is a product rich in vitamins, essential amino acids, and polyunsaturated fatty acids (PUFAs) for some species. Fish product intake supplies consumer needs for a healthier diet, mainly because PUFAs are related to a reduced risk of developing chronic diseases in the cardiovascular, immune, and cognitive systems (Chen et al., 2022).

Despite this promising context for the production chain of seafood and freshwater fish, challenges regarding their hygienic-sanitary safety persist, and the shelf life of fish products is a key point for the industry. The intrinsic characteristics of these products support the acceleration of autolysis, lipid oxidation, and other spoilage metabolites, and the fish muscle structure favors microbial growth (Reitznerová et al., 2017; Sheng & Wang, 2020; Nie et al., 2022).

For these reasons, some products from the fishing industry end up being devalued, as the main mechanism to delay the deterioration reactions in the sector is the cold chain, which is not always present or adequate to guarantee safety, control, and prolonged shelf life. Although the food industry uses chemical or synthetic preservatives to control spoilage reactions, several studies have raised relevant questions about the risks of excessive use of synthetic additives (Wang & Kannan, 2019; Liu & Mabury, 2020; Xu, et al., 2021). An alternative is the application of natural compounds such as essential oils (EOs) and plant extracts with bioactive activity. Several studies have pointed out numerous groups of plants with antioxidant and antimicrobial potential in recent decades. The positive effects are mainly related to the presence of secondary metabolites, such as phenolic acids, which show interaction with the lipid and protein composition of animal products, with significant results in the extension of shelf life (Basavegowda & Baek, 2021; Huang et al., 2021). Considering that some fish consumers tend to be more attentive to safety and health issues, the combination of natural products in fish conservation can meet a range of consumers looking for clean-label products, also corroborating the reduction of agri-food industry waste.

Previous studies have already provided insights into the application of active natural compounds in fish products (Maqssod et al., 2012; Hassoun & Çoban, 2017; Shahidi & Hossain, 2022); however, an evaluation of the methods of obtaining the compounds and application tests were not compiled to assess trends and challenges. In addition, the combination of a systematic review and bibliometric analysis allows evaluation of the evolution of specific themes through micro- and macrostructural views of topics based on a visual presentation of analytical data that constitute literary research (Ellegard & Wallin, 2015).

The present study carried out a systematic review and used VOSviewer software to achieve the bibliometric objectives. This review discusses, I: significant results for the topic, such as coauthorship, metrics by country, and keywords, II: investigation of trends for extraction of bioactive compounds from plants with potential application in fisheries products, and III: results on prolongation of the shelf life of fish products with the addition of active plant compounds.

2.2. Chosen research strategy and methodology

The research developed followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) strategy (Moher et al., 2009), following the checklist, which contains 27 items and a flowchart for the search and selection of scientific papers. This strategy allowed greater reliability in the systematic search based on 4 phases: identification, selection, eligibility, and inclusion (Figure 1). Searches were performed on the Web of Science™ (WoS) in December 2021 using the terms "((ALL= (fish) OR ALL= (fish product)) AND ALL= (natural extract) AND (ALL= (shelf life) OR ALL= (conservation)))". The results included the title, abstract, and keywords. It is noteworthy that the year filter was not used so that the results would contain all the scientific papers present in the database. The search was constructed in 3 phases: 1st phase: the construction of the search term, 2nd phase: reading the title and abstract, and 3rd phase: reading the article, identifying aspects of interest, and establishing review analysis. Inclusion criteria were I: complete manuscripts, II: manuscripts focusing on plant compounds, and III: manuscripts with test applications on fish. Exclusion criteria, I: publications of abstracts, II: review articles, III: studies that did not show the application of plant compounds in fish products. After applying the criteria, a list of 68 scientific papers was compiled for further analysis.

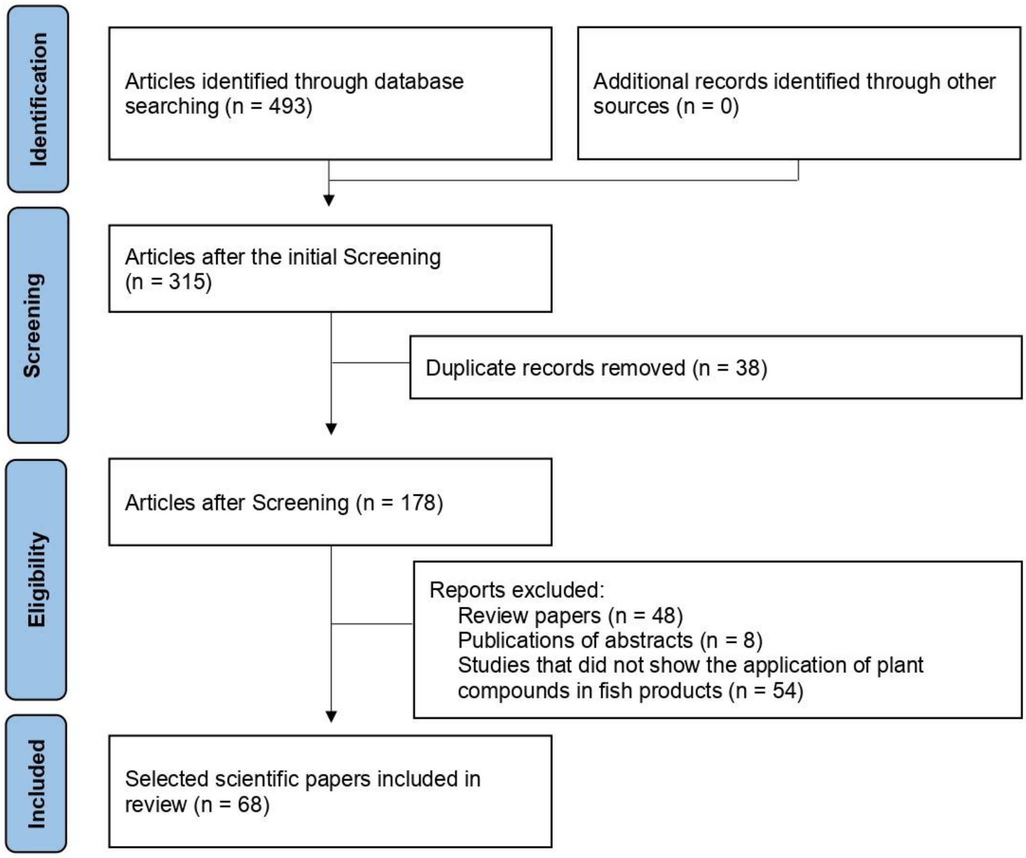

Figure 1. Results of a systematic literature search on the application of plant bioactive compounds to extend shelf life in fish products from 2005 to 2021 using the PRISMA flow diagram.

2.3. Bibliometric methodology

Bibliometric analysis provides a quantitative discussion of a given dataset to assess emerging trends and can provide relevant information on specific topics, such as structural insight into countries' performance, coauthorship, and co-occurrence of keywords. The data extracted directly from the Web of Science™ (WoS) were processed in VOSviewer software (Version 1.6.18) to build bibliographic mapping as described in the studies of Donthu et al. (2021) and Melo et al. (2021).

2.4. Results

2.4.1. Bibliometric analyses

The first study on the feasibility of applying plant extracts in fish products at WoS™ was in 2005, and the number of publications was significantly higher from 2015 onward, highlighting 2019. Twenty-four countries contributed to the 68 scientific papers selected based on the criteria of this review (Figure 2); among the top 5 in the number of documents are Iran (Number of documents: 16), Spain (Number of documents: 15), China (Number of documents: 10), and Turkey and Brazil (Number of documents: 8). Based on WoS™, it is possible to observe that the topic has been investigated on all continents, since fish are an important food component for different cultures, and the strategy of using natural compounds has been widely disseminated in recent decades. Despite the highest number of publications, Iran (average citation rate: 10.47%) ranks 4th in terms of the percentage of citations, behind China (average citation rate: 27.66%), Spain (average citation rate: 26%), and Turkey (average citation rate: 11.65%).

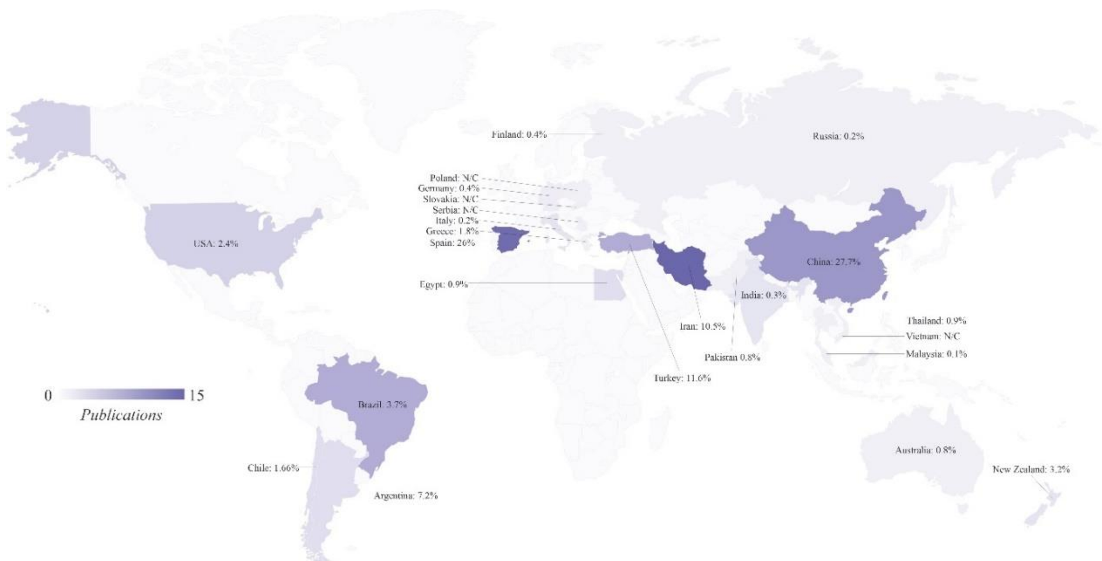

Figure 2. Publications by country associated with the 68 scientific articles identified on the application of plant compounds in fish products from 2005 to 2021 and their respective participation in the percentage of citations.

Although Brazil is among the main contributors to the number of scientific papers, its average citation rate was low (average citation rate: 3.7%), which may be related to the choice for publication, since only 1 Brazilian article was published among one of the 5 most cited journals.

The coauthorship interaction between countries was low across the 24 countries, with only 13 countries identified with coauthorship collaborations. Despite being the largest author in the number of publications, Iran only interacted with New Zealand. However, Spain being the second largest contributor in the number of publications is the one that most maintains coauthorship interactions with other countries (Average interaction rate: 11), followed by the USA (Average interaction rate: 5) and China (Average interaction rate: 3). In addition, it is possible to observe that the trend of publications on the subject is more evident among countries from 2015 onward.

The lack of interaction between the others may be related to the compounds analyzed and the fish species investigated, indicating a local approach to the origin of raw materials. However, studies by Raeisi et al. (2017) and Ucak et al. (2018) evaluated the application of garlic (Allium sp.) in freshwater species, and studies by Kunová et al. (2021), Joukar et al. (2017), and Valizadeh et al. (2020) evaluated the application of Cinnamomum sp. in rainbow trout (Oncorhynchus mykiss), thus constituting a relevant existence of future scientific partnerships between countries to improve the theme. Regarding the citations, the bibliometric analysis revealed that for the set of scientific articles investigated, 1708 citations were identified among the 68 scientific articles, and the 5 most cited corresponded to 61.77% of the total citations.

The bibliographic survey allowed us to identify 442 different keywords used among the analyzed scientific articles. This analysis allows us to identify trends since the keywords are vital components for the bibliographic research, as they indicate themes about the research field (Ellegaard & Wallin, 2015). This dispersion is mainly related to the different compounds studied and the application approach among the different fish. The keywords frequently adopted (≥ 10) were Quality; Shelf life; Fish; Lipid oxidation; Antioxidant; Antioxidant activity and Storage. The co-occurrence analysis presented in Figure 3 shows the network of keywords and their links, the dimension of the circle that represents the highest value for the words within the analyzed topic. In addition, it is possible to observe that despite the larger groups being the most recounted, there is a greater relationship between the citation index and reddish color keywords. This result can help future research, allowing authors to have more assertive decision-making in choosing keywords on the topic.

It is still possible to observe from the keywords the trend in the evaluation of plant compounds, with the potential antioxidant effect on the lipid composition of fish being the main investigation component.

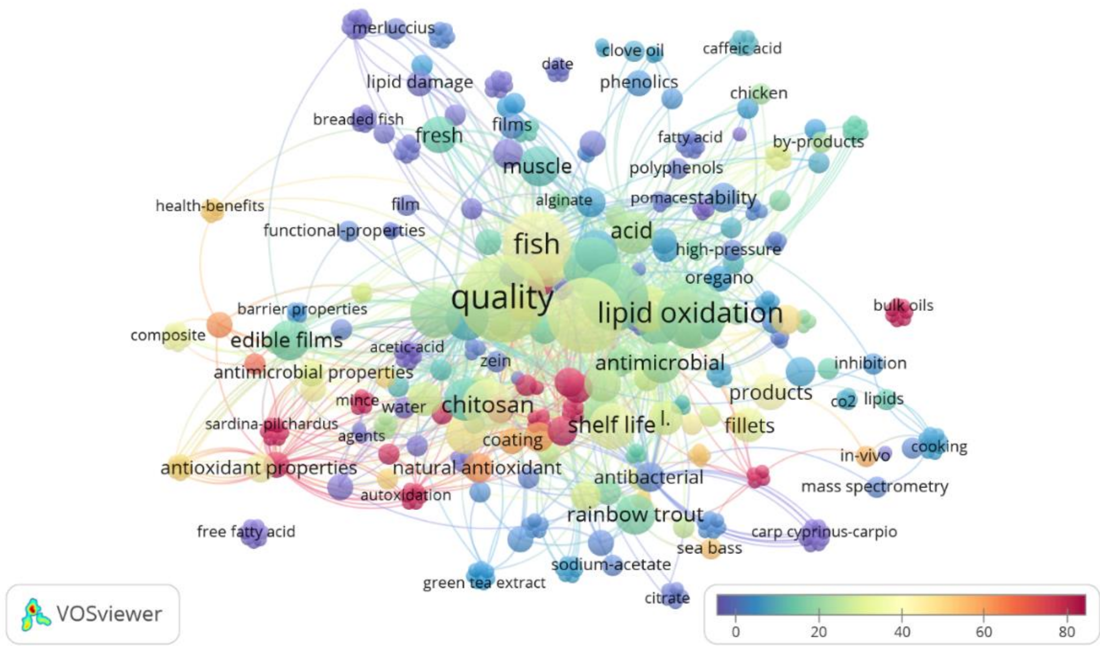

Figure 3. Overlay visualization map of co-occurrence (keywords/number of citations) in publications on the application of plant compounds in fish products from 2005 to 2021.

2.4.2. Plant bioactive compounds

Active plant compounds are available in various groups, such as fruits, vegetables, cereals, herbs, and algae. The use of these compounds can benefit the food industry and consumers and enable the reuse of byproducts, resulting in economic gains and reduced waste, since these compounds make up different parts of vegetables, such as pomace, peel, and seeds. These active biocompounds are mainly derived from secondary compounds, such as phenolic compounds, and can be applied to food products in the form of essential oils (EOs), extracts, or in natura or through other methods that aim to optimize their application.

The application of plant extracts or EOs to increase the shelf life of fish products has been evidenced in recent years. At the time of this research, more than 50 plant species were evaluated among the scientific papers in the WoS™ database with the potential to prevent the deterioration of products with the ability to prolong shelf life (Table 1).

2.4.1. Extraction methods applied to obtain bioactive compounds from plants

Among the scientific papers investigated, more than 11 different methods/solvents were used for the extraction of bioactive compounds. This approach to the extraction methods used is potentially significant to support future studies aimed at optimizing the extraction process, especially in the investigation of the feasibility of replacing potentially harmful organic solvents with green solvents, that is, those that are not toxic, to allow the use of active plant compounds (Pateiro et al., 2021).

Among the most commonly used solvents, ethanol was used in 34% of the scientific papers, followed by distilled water (dh_2o), which was present in 25%. In studies with garlic extract (*Allium sp.*) An aqueous extraction of 1:10 (sample/dh_2o) was used in tests with fillets of Atlantic herring (*Clupea harengus*) treated with 10% (w/w) of the extract, while an ethanolic extract of 1:10 (sample/ethanol) was applied to the fillet of rainbow trout (*Oncorhynchus mykiss*) at a concentration of 3% (w/w). The ethanolic extract of garlic, even at lower concentrations, indicated a better result in the extension of the shelf life of the fillets compared to the study with aqueous extracts (Raeisi et al., 2016; Ahmed et al., 2019), reinforcing the efficiency of organic solvents for better extraction of bioactive compounds.

Another result with aqueous and alcoholic extracts was evidenced in studies with rosemary (*Rosmarinus officinalis*). The study proposed ethanolic extraction 2:10 (sample/ethanol) of rosemary samples, with the application of 10 g (p/p) on European pilchard (*Sardina pilchardus*) fillets that showed a TVB-N value of 20.5 mg ($N/100g^{-1}$), while the study with aqueous extraction 0.75:10 (sample/dh_2o) and concentration of 10 mL (v/p) applied to the fillet of common carp (*Cyprinus carpio*) presented a value of 30 mg ($N/100g^{-1}$), both results after 10 days under refrigeration (Kenar et al., 2010; Abdeldaiem et al., 2017).

Table 1. Investigated plant species with the potential to extend the shelf life of fish products in scientific papers from 2005 to 2021 in the Web of Science™ Core Collection.

Plant species	Used part	Extraction method	Related compound	Reference
Achyroclines satureiodes	Leaves	Ethanol and dh₂o	Phenolic compounds	Lima et al. (2017)
Actinidia deliciosa	Aerial parts or peel	ddh₂o	Phenolic compounds	Abdel-Wahab et al. (2020)
Allium sp.	Peel, fruit or leaves	dh₂o and ethanol	Phenolic compounds and flavonoids	Ucak et al. (2018); Ahmed et al. (2019); Raeisi et al. (2017); Raeisi et al. (2016)
Alpinia zerumbet	Leaves	Hydrodistillation	Phenolic compounds	Sousa et al. (2019)
Bifurcaria bifurcata	Aerial parts	dh₂o and ethanol	Polyphenols	Miranda et al. (2021); Miranda et al. (2016)
Brassica oleracea	Leaves	dh₂o	Polyphenols	Ali et al. (2019)
Camellia sinensis	Leaves	Ethanol	Phenolic compounds	Özogul & Ucar (2013)
Campomanesia xanthocarpa	Leaves	In natura	Phenolic compounds	Cristofel et al. (2021)
Carya illinoiensi	Fruit or EO	In natura and commercial	Phenolic compounds, eugenol, and ß-caryophyllene	Villasante et al. (2019); Coban & Patir (2013)
Chenopodium quinoa	Grains	Ethanol	Phenolic compounds	Miranda et al. (2018)
Chlorella vulgaris	Aerial parts	dh₂o	Methyl linolenate, dioctylamine, and monoterpenoids	Özogul et al. (2021)
Cinnamomum sp.	EO, peel or aerial parts	Commercial, hydrodistillation, and dh₂o	Monoterpenoids (linalool and α-limonene), phenolic compounds, and flavonoids	Kunová, et al. (2021); Valizadeh et al. (2020); Haghighi & Yazdanpanah (2020); Chuesiang, Sanguandeekul & Siripatrawan (2020); Joukar et al. (2017); Abdeldaiem, Ali & Ramadan (2017)
Citrus sp.	EO or peel	Commercial, ethanol, and methanol	Monoterpenoids (linalool and α-limonene) and phenolic compounds	Kunová, et al. (2021); GhareAghaji et al. (2021); Mayeli et al. (2019)
Crithmum maritimun	Aerial parts	dh₂o	Phenolic compounds	Rico et al. (2020)
Elettaria cardamomum	Aerial parts	Hydrodistillation	Phenolic compounds	Abdeldaiem, Ali & Ramadan (2017)
Eryngium caucasicum	Leaves	Ethanol	Monoterpenoids	Raeisi et al. (2017)
Eugenia caryophyllata	EO	Commercial	Eugenol and ß-caryophyllene	Coban & Patir (2013)
Foeniculum vulgare	Leaves or aerial parts	Ethanol, hydrodistillation, and steam distillation	Polyphenols and phenolic compounds	Bagheri et al. (2016); Abdeldaiem, Ali & Ramadan (2017); Sayyari et al. (2021)

Fucus spiralis	Leaves	Lyophilization	Polyphenols	Trigo et al. (2022)
Garcinia sp.	Peel	Dh_2o	Phenolic compounds	Apang et al. (2020)
Helichrysum sp.	Leaves	Ethanol	Phenolic compounds	Özogul, Kus & Kuley (2013)
Hibiscus sabdariffa	Fruit	In natura	Phenolic compounds	Villasante et al. (2019)
Hordeum sp.	Peel	Hydrolysis and methanol	Phenolic compounds	Abreu et al. (2010)
Illicium verum	EO	Commercial	Monoterpenoids (carvacrol and thymol) and phenylpropanoid (trans-anethole)	Huang et al. (2018)
Laurus nobilis	Leaves	Hydrodistillation and ethanol	Phenolic compounds	Özogul et al. (2017); Özogul & Ucar (2013)
Lavandula sp.	Aerial parts	n-hexane	Phenolic compounds	Delfino et al. (2021)
Majorana syriaca	Leaves	Soxhlet (ethyl acetate)	Phenolic compounds and flavonoids	Al-Bandak et al. (2009)
Mentha aquatica	Aerial parts	Hydrodistillation and ethanol	Phenolic compounds	Amoli et al. (2019)
Moringa oleifera	Leaves	n-hexane	Phenolic compounds	Delfino et al. (2021)
Musa sp.	Peel	dh_2o	Polyphenols	Ali et al. (2019)
Olea europaea	Leaves or fruit	Ethanol, dh_2o, acetone, and centrifuge	Phenolic compounds	Albertos et al. (2017); Martinez et al. (2019); Panza et al. (2020)
Origanum vulgare	EO or leaves	Commercial and ethanol Co_2	Monoterpenoids (carvacrol and thymol), phenylpropanoid (trans-anethole) phenolic compounds, and polyphenols	Huang et al. (2018); Tarvainen et al. (2016); Cardoso et al. (2017)
Padina tetrastromatica	Aerial parts	Methanol	Phenolic compounds	Lekshmi et al. (2021)
Perilla frutescens	Leaves	Ethanol	Polyphenols	Zhao et al. (2019)
Phoenix dactylifera	Fruit	dh_2o	Phenolic compounds	Seifzadeh & Khorasgani (2020)
Pimpinella affinis	Aerial parts or fruit	Ethanol and hydrodistillation	Phenolic compounds and monoterpenoids	Esmaeli et al. (2019); Ariaii et al. (2015)
Pistacia atlantica	Fruit	Steam distillation	Phenolic compounds	Hassanzadazar et al. (2021)
Punica granatum	Peel	Ethanol and acetone	Phenolic compounds	Khodanazary (2019); Martinez et al. (2019)
Rosmarinus officinalis	Leaves, aerial parts, or EO	Ethanol, CO_2, hydrodistillation, commercial and acetone	Phenolic compounds and Polyphenols	Tarvainen et al. (2016); Özogul, Kuley & Kenar (2011); Kenar, Özogul & Kuley (2010); Abdeldaiem, Ali & Ramadan (2017); Martinez et

				al. (2019); Özogul et al. (2017); Li et al. (2012a); Li et al. (2012b); Gao et al. (2014)
Salvia officinalis	Leaves, aerial parts, or peel	Ethanol, hydrodistillation, and ddh₂o	Phenolic compounds	Özogul, Kuley & Kenar (2011); Kenar, Özogul & Kuley (2010); Özogul et al. (2017); Abdel-Wahab et al. (2020); Özogul & Ucar (2013)
Schinus terebinthifolius	Fruit	In natura	Phenolic compounds	Fortunato et al. (2019)
Spirulina platensis	Lyophilized powder or aerial parts	Commercial and dh₂o	Phycobiliprotein, methyl linolenate, Dioctylamine and monoterpenoids	Stejskal et al. (2020); Özogul et al. (2021)
Stevia rebaudiana	Leaves	Ethanol and dh₂o	Phenolic compounds	Ortiz-Viedma et al. (2017)
Syzygium aromaticum	EO, aerial parts, peel or flower buds	Commercial, ddh₂o, and hydrodistillation	Phenolic compounds. Phenylpropanoid (eugenol) and monoterpenoids (thymol)	Mubarak, Othman & Karim (2019); Echeverria et al. (2018); Li et al. (2017); Salgado et al. (2013); Abdel-Wahab et al. (2020); Dehghani et al. (2018); Shi et al. (2014)
Thymus sp.	EO	Commercial and hydrodistillation	Monoterpenoids (carvacrol and thymol), phenylpropanoid (trans-anethole), and phenolic compounds	Huang et al. (2018); Özogul et al. (2017)
Trachyspermum ammi	Seed	Ethanol	Phenolic compounds	Raeisi et al. (2016)
Viscum album	Leaves	Ethanol	Phenolic compounds	Özogul, Kus & Kuley (2013)
Vitis sp.	Peel, seed, or pomace	Methanol, dh₂o, and ethanol	Phenolic compounds and flavonoids	Simoes et al. (2019); Gai et al. (2015); Pazos et al. (2005); Shi et al. (2014)
Zataria multiflora	Flower buds or aerial parts	Hydrodistillation	Phenolic compounds, phenylpropanoid (eugenol), and monoterpenoids (thymol)	Joukar et al. (2017); Dehghani et al. (2018)
Zingiber officinale	Rhizomes or EO	dh₂o, hydrodistillation and commercial	Monoterpenoids, phenolic compounds, and polyphenols	Minh (2021); Cai, Wang & Cao (2020); Ahmed et al. (2019); Mattje et al. (2019)

Other organic solvents were used for phenolic extraction. Peel and seed extracts of muscadine grape (*Vitis rotundifolia*), obtained with methanol/water solution (70:30 v/v), reduced the value of TBARS and PV in fresh Atlantic salmon (*Salmo salar*) after 192 hours of refrigeration storage (4 °C); however, a study with moringa extract (*Moringa oleifera*) and lavender (*Lavandula sp.*) obtained with a Soxhlet extractor using n-hexane (1:10) had no significant effect on reducing the lipid oxidation of Nile tilapia (*Oreochromis niloticus*) fish burgers (Simoes et al., 2019; Delfino et al., 2021).

2.4.2. Bioactive plant residues are an even more sustainable alternative to extend the shelf life of fish products

In addition to the main objective of evaluating the positive effects on the extension of the shelf life of fish, 12 scientific papers showed the possibility of converting residues, mainly the peels and seeds of vegetables and fruits, into active byproducts. The study using the extract from the peel of garcinia (*Garcinia cambogia*) showed a total phenolic content of 4.17 mg (GAE/g) and DPPH radical scavenging activity of 36.88 (% inhibition). The study also showed a reduction of approximately 50% for the TVB-N value and 40% for the TBARS value after 15 days of refrigeration in Indian mackerel fillets (*Rastrelliger kanagurta*) (Apang et al., 2020).

Similar results on the potential of byproducts as preservatives were evaluated for bitter orange (*Citrus aurantium*), the values of DPPH radical scavenging activity were 59.38 (% inhibition) for the ethanolic extract, and the assay with the fillet coated with zein rainbow trout (*Oncorhynchus mykiss*) with 2% ethanolic extract and vacuum packaging showed a reduction of approximately 50% in the value of TVB-N and TBA after 12 days of cooling of the control (Mayeli et al., 2019).

Studies that evaluated the application of byproducts in conventional products also showed significant responses on the bioactive capacity. Tests with minced meat of marine and freshwater fish with extracts of the byproduct of grape processing (*Vitis vinifera*) were satisfactory to reduce lipid oxidation and prolong the shelf life of the products. In the evaluation with minced mackerel (*Scomber scombrus),* the reduction of malonaldehyde value was higher than 50% compared to the control (without antioxidant) after 80 days in freezing (-10 °C), and in the evaluation with chopped rainbow trout (*Oncorhynchus mykiss*), the TBARS value was 0.70 mg (MDA/kg^{-1}) for application of 1% red grape pomace extract, while the control showed a value of 2.86

mg (MDA/kg^{-1}) after 6 days under refrigeration (Pazos et al., 2005; Gai et al., 2014). Therefore, the foreign idea of using plant byproducts as natural agents for the conservation of fish products and addressing the economically important issue of product quality corroborates the mitigation of waste from the agri-food industry.

2.4.3. Role of phenolic compounds on shelf life extension

Based on the scientific articles evaluated in this review, it was possible to observe that the objective is not always to investigate which active compound is responsible for possible beneficial responses to the shelf life of fish products. In 40 studies, the feasibility of applying the extract or EOs was justified through the analysis of total phenolic compounds, showing that most authors seek to evaluate the application potential only through the composition of total phenolic compounds since these compounds represent the most abundant group among the secondary metabolites in plants (Ayad & Akkal, 2019).

Phenolic compounds are phytochemicals formed by hydroxylated aromatic rings directly linked to a phenyl group. These compounds, despite not being evidenced as important dietary nutrients, have protective active properties, such as antibacterial, anti-inflammatory, and antioxidant activities (De La Rosa, 2019). Depending on the chemical structure, these compounds can be distributed into some classes that include flavonoids, phenolic acids, tannins, and others, which can capture free radicals in the initiation and propagation stages of lipid oxidation reactions, delaying the deterioration activities of the products (Figure 4). Phenolic compounds, therefore, are applicable as preservatives, mainly because they present a stable molecular structure in the form of an aromatic ring; however, it is necessary to evaluate which metabolites are present in the phenolic composition of plants, some of which may have potential toxicity.

Some of the scientific articles provided a better description of the investigated compounds, with a detailed approach on metabolites present and other preservative effects, such as phenylpropanoids, which are metabolites derived from phenylalanine and have effects on enzymatic reactions, such as lipid oxidation, and show significant results in the control of microbial cultures (Soledade et al., 2010). According to Echeverria et al. (2018), the application of a film enriched with phenylpropanoid in a test with 0.5 ml (w/v) of the EOs of clove (*Syzygium* *aromaticum*) in the fillet of Atlantic bluefin tuna (*Thunnus thynnus*) obtained positive effects on shelf life with a reduction in microbial and spoilage chemical reactions.

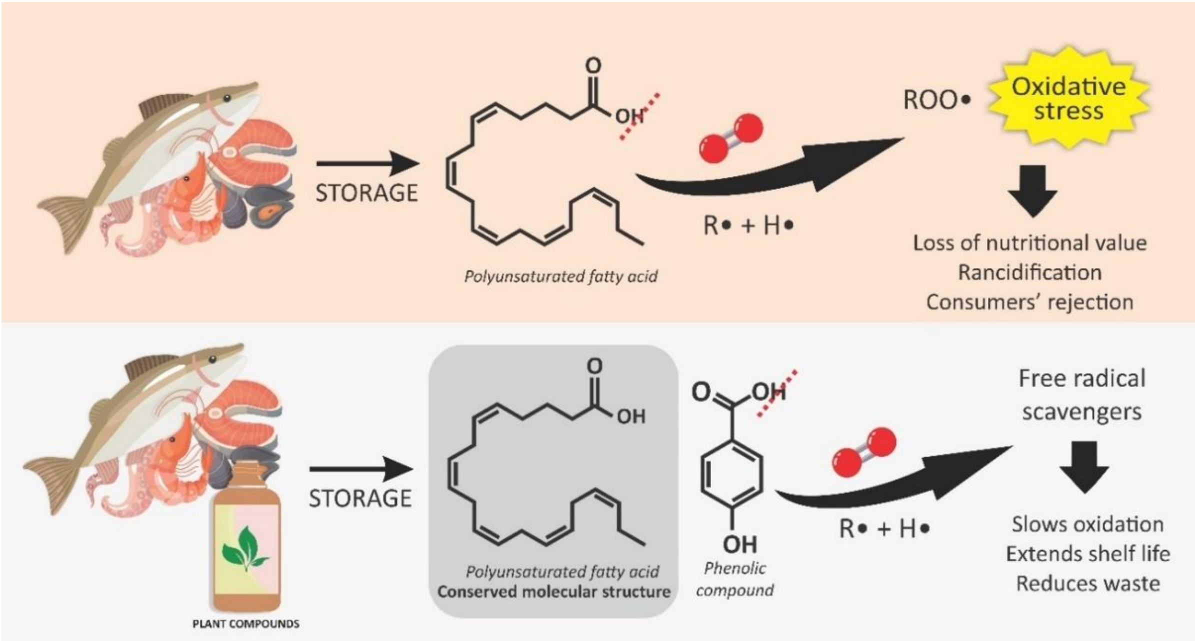

Figure 4. Simplified processes of lipid oxidation. Impacts on the quality of fish products Interaction of plant phenolic compounds to scavenge free radicals (R•) and prolong shelf life.

Other groups of compounds have been identified for application, such as polyphenols that have a structure composed of one or more aromatic rings with several hydroxyl groups and are more reactive in inhibiting oxidative enzymes with a neutralizing effect on free radicals (Maqsood et al., 2012), such as flavonoids that interact with hydroxyl and peroxyl groups and can eliminate reactive oxygen species (ROS) (Shen et al., 2022). Assays with polyphenolic extracts of common grapevine (*Vitis vinifera*) and perilla (*Perilla frutescens*) at concentrations of up to 3% in the fish burger and surimi showed a reduction in oxidative activity and a decrease in the formation of total volatiles (Gai et al., 2015; Zhao et al., 2019). Monoterpenoids comprise a class of terpenoids composed of unsaturated hydrocarbons or functional groups such as alcohols, aldehydes, and ketones. Some of these compounds, such as α-terpineol, limonene, and thymol, are commonly used as aromatic additives (Ludwiczuk et al., 2017). In an assay with 0.5% (v/v) terpenoid EOs of sweet oranges (*Citrus sinensis*) on rainbow trout fillets (*Oncorhynchus mykiss*) under refrigeration, a reduction in TVB-N and TBA values was observed (GhareAghaji et al., 2021).

With the grouping of review responses, it was possible to verify that, depending on the extraction method and application concentrations, the effects are different, even in studies that investigated the potential of the same plant or applied to the same species of fish. Furthermore, a more in-depth evaluation of the compounds studied allows a better exploration of the results, since the difference in the chemical composition between the metabolites tends to express different effects on the deteriorating factors.

Another line of research proposed is the combination of different active compounds with synergistic capacity to improve application efficiency, using a shelf life test of rainbow trout fillets (*Oncorhynchus mykiss*) under refrigeration coated with EOs of zataria (*Zataria multiflora*) (rich in eugenol) and clove (*Syzygium aromaticum*) (rich in thymol), where the values of TBARS and TVB-N were lower in the treatment with the mixture of EOs compared to the treatment with pure EOs (Dehghani et al., 2018). Studies applied as response surface models can be used to better understand the synergistic effects since different compounds act on different metabolites, and the combination of these can provide better results in extending the shelf life of fish products.

Table 2. Investigated methods to study the shelf life of fish products with the application of plant species with active compounds in scientific papers from 2005 to 2021 in the Web of Science™ Core Collection.

Plant species	Application method	Application concentration	Product tested	Storage study	Evidenced effect	Reference
Achyroclines satureiodes	Mixed	0 - 0.75% (v/w)	Sausages	Refrigeration	Antioxidant	Lima et al. (2017)
Actinidia deliciosa	Mixed	0.1 - 0.5% (v/v)	Fish finger	Refrigeration	Antimicrobial and antioxidant	Abdel-Wahab et al. (2020)
Allium sp.	Immersion and mixed	2 - 10% (v/v)	Fresh fillet	Refrigeration	Antimicrobial, antioxidant, and inhibitory to the formation of total volatiles	Ucak et al. (2018); Ahmed et al. (2019); Raeisi et al. (2017); Raeisi et al. (2016)
Alpinia zerumbet	Immersion	0.75 - 1.5% (v/v)	Fresh fillet	Refrigeration	Inhibitory to the formation of total volatiles	Sousa et al. (2019)
Bifurcaria bifurcata	Ice coating	0.67 - 2.5 g/L	Whole fish	Refrigeration	Antimicrobial and inhibitory effects on the formation of fluorescent compounds	Miranda et al. (2021); Miranda et al. (2016)
Brassica oleracea	Mixed	0 - 1.5% (v/w)	Meatballs	Refrigeration and freezing	Antimicrobial and antioxidant	Ali et al. (2019)
Camellia sinensis	Mixed	0.3 - 0.6% (w/w)	Fish burger	Refrigeration	Antioxidant and inhibitory to the formation of total volatiles	Özogul & Ucar (2013)
Campomanesia xanthocarpa	Mixed	5% (w/v)	Fish burger	Freezing	Inhibitory to the formation of total volatiles	Cristofel et al. (2021)
Carya illinoiensi	Mixed and dip coating	0.1 - 10% (w/v)	Fish patties and smoked fillet	Refrigeration	Antimicrobial, antioxidant, and inhibitory to the formation of total volatiles	Villasante et al. (2019); Coban & Patir (2013)
Chenopodium quinoa	Ice coating	0.05 - 0.20 g/L	Whole fish	Refrigeration	Antimicrobial, antioxidant, and inhibitory to the formation of total volatiles	Miranda et al. (2018)
Chlorella vulgaris	Immersion	10 g/L	Fresh fillet	Refrigeration	Antimicrobial and antioxidant	Özogul et al. (2021)
Cinnamomum sp.	Immersion, coating, dip	0.25 - 1.5% (w/v), 0 - 11429 mg/L or 10 mL/kg	Fish burger, fresh fillet and fish finger	Refrigeration	Antioxidant and antimicrobial	Kunová, et al. (2021); Valizadeh et al. (2020); Haghighi & Yazdanpanah

	coating, and mixed					(2020); Chuesiang, Sanguandeekul & Siripatrawan (2020); Joukar et al. (2017); Abdeldaiem, Ali & Ramadan (2017)
Citrus sp.	Immersion and dip coating	0.25 - 2% (w/v)	Fresh fillet	Refrigeration	Antioxidant, antimicrobial	Kunová, et al. (2021); GhareAghaji et al. (2021); Mayeli et al. (2019)
Crithmum maritimun	Ice coating	12.5% (w/v)	Fish burger	Refrigeration	Antimicrobial and antioxidant	Rico et al. (2020)
Elettaria cardamomum	Mixed	10 mL/kg	Fish finger	Refrigeration	Antioxidant	Abdeldaiem, Ali & Ramadan (2017)
Eryngium caucasicum	Immersion	2 - 4% (v/v)	Fresh fillet	Refrigeration	Antimicrobial and antioxidant	Raeisi et al. (2017)
Eugenia caryophyllata	Dip coating	0.1 - 1% (w/v)	Smoked fillet	Refrigeration	Antimicrobial and inhibitory to the formation of total volatiles	Coban & Patir (2013)
Foeniculum vulgare	Mixed and ice coating	0.3 - 2% (w/v) or 10 mL/kg)	Fish patties, minced fish, and fish finger	Refrigeration	Antimicrobial and inhibitory to the formation of total volatiles	Bagheri et al. (2016); Abdeldaiem, Ali & Ramadan (2017); Sayyari et al. (2021)
Fucus spiralis	Coating	5% (w/v)	Fresh fillet	Refrigeration	Antioxidant	Trigo et al. (2022)
Garcinia sp.	Ice coating	500 a 750 mg/L	Fresh fillet	Refrigeration	Antioxidant	Apang et al. (2020)
Helichrysum sp.	Immersion	5 g/L	Fresh fillet	Refrigeration	Antioxidant	Özogul, Kus & Kuley (2013)
Hibiscus sabdariffa	Mixed	5 - 10% (w/v)	Fish patties	Refrigeration	Antimicrobial	Villasante et al. (2019)
Hordeum sp.	Coating	7 - 24 mg/dm^2	Fresh fillet	Freezing	Antioxidant	Abreu et al. (2010)
Illicium verum	Immersion	0.1% (v/v)	Fresh fillet	Refrigeration	Antimicrobial	Huang et al. (2018)
Laurus nobilis	Immersion	4% (w/v)	Fresh fillet	Refrigeration	Antimicrobial and antioxidant	Özogul et al. (2017); Özogul & Ucar (2013)
Lavandula sp.	Mixed	0.2% (w/w)	Fish burger	Refrigeration	Antioxidant	Delfino et al. (2021)

Majorana syriaca	Immersion	0 - 3900 ppm	Fresh fillet	Refrigeration	Antioxidant	Al-Bandak et al. (2009)
Mentha aquatica	Immersion	0.5 a 1% (v/v)	Whole fish	Refrigeration	Antioxidant	Amoli et al. (2019)
Moringa oleifera	Mixed	0.2% (w/w)	Fish burger	Refrigeration	Antioxidant	Delfino et al. (2021)
Musa sp.	Mixed	0 - 1.5% (v/w)	Meatballs	Refrigeration and freezing	Antimicrobial and antioxidant	Ali et al. (2019)
Olea europaea	Coating and mixed	0 - 20% (w/v) or 200 ppm	Smoked fillet, fish finger, and fish patties	Room temperature and refrigeration	Antimicrobial, antioxidant, and inhibitory to the formation of total volatiles	Albertos et al. (2017); Martinez et al. (2019); Panza et al. (2020)
Origanum vulgare	Immersion, mixed and dip coating	0.1 - 1% (w/v)	Fresh fillet and fish patties	Refrigeration	Antimicrobial and inhibitory to the formation of total volatiles	Huang et al. (2018); Tarvainen et al. (2016); Cardoso et al. (2017)
Padina tetrastromatica	Immersion	0.5 - 2% (v/v)	Whole fish	Refrigeration	Antimicrobial and antioxidant	Lekshmi et al. (2021)
Perilla frutescens	Mixed	0 - 0.3 g/kg	Surimi	Refrigeration	Antioxidant and inhibitory to the formation of total volatiles	Zhao et al. (2019)
Phoenix dactylifera	Immersion	3% (v/v)	Fish burger	Refrigeration	Antioxidant	Seifzadeh & Khorasgani (2020)
Pimpinella affinis	Dip coating	1 - 2% (w/v)	Fresh fillet	Refrigeration	Antioxidant	Esmaeli et al. (2019); Ariaii et al. (2015)
Pistacia atlantica	Mixed	0.125 - 0.5 (v/w)	Minced fish	Refrigeration	Inhibitory to the formation of total volatiles	Hassanzadazar et al. (2021)
Punica granatum	Coating and mixed	1.5% (w/v) or 200 ppm	Fresh fillet and fish patties	Refrigeration	Antioxidant	Khodanazary (2019); Martinez et al. (2019)
Rosmarinus officinalis	Mixed and immersion	10 mL/kg, 200 ppm or 1 - 4% (w/v)	Fish finger, fish patties, and fresh fillet	Refrigeration	Antimicrobial and antioxidant	Tarvainen et al. (2016); Özogul, Kuley & Kenar (2011); Kenar, Özogul & Kuley (2010); Abdeldaiem, Ali & Ramadan (2017); Martinez et al. (2019); Özogul et al. (2017); Li et al. (2012a); Li et al. (2012b); Gao et al. (2014)

Salvia officinalis	Immersion and mixed	0.1 - 4% (w/v)	Fresh fillet and fish finger	Refrigeration	Antimicrobial and antioxidant	Özogul, Kuley & Kenar (2011); Kenar, Özogul & Kuley (2010); Özogul et al. (2017); Abdel-Wahab et al. (2020); Özogul & Ucar (2013)
Schinus terebinthifolius	Mixed	100 ppm	Fish patties	Refrigeration	Antioxidant	Fortunato et al. (2019)
Spirulina platensis	Coating and immersion	10 - 20 g/L	Fresh fillet	Refrigeration	Antimicrobial, antioxidant, and inhibitory to the formation of total volatiles	Stejskal et al. (2020); Özogul et al. (2021)
Stevia rebaudiana	Mixed	2 - 4.4% (w/v)	Fish patties	Refrigeration	Biochemical reactions	Ortiz-Viedma et al. (2017)
Syzygium aromaticum	Immersion, coating and mixed	0.1 - 2% (w/v) or 0.75 mL/g	Fresh fillet, whole fish, fish patties, and fish finger	Refrigeration	Antimicrobial, antioxidant, and inhibitory to the formation of total volatiles	Mubarak, Othman & Karim (2019); Echeverria et al. (2018); Li et al. (2017); Salgado et al. (2013); Abdel-Wahab et al. (2020); Dehghani et al. (2018); Shi et al. (2014)
Thymus sp.	Immersion	0.1 - 4% (w/v)	Fresh fillet	Refrigeration	Antimicrobial and antioxidant	Huang et al. (2018); Özogul et al. (2017)
Trachyspermum ammi	Dip coating	1.5- 3.0% (w/w)	Fresh fillet	Refrigeration	Antioxidant	Raeisi et al. (2016)
Viscum album	Immersion	5 g/L	Fresh fillet	Refrigeration	Antioxidant	Özogul, Kus & Kuley (2013)
Vitis sp.	Immersion and mixed	1 - 3% (v/v)	Fresh fillet and fish burger	Refrigeration	Antimicrobial and antioxidant	Simoes et al. (2019); Gai et al. (2015); Pazos et al. (2005); Shi et al. (2014)
Zataria multiflora	Dip coating and immersion	0.5 - 2% (w/v)	Fresh fillet	Refrigeration	Antioxidant	Joukar et al. (2017); Dehghani et al. (2018)
Zingiber officinale	Immersion, coating, and mixed	0.2 - 1% (w/v)	Fresh fillet	Refrigeration	Antioxidant and inhibitory to the formation of total volatiles	Minh (2021); Cai, Wang & Cao (2020); Ahmed et al. (2019); Mattje et al. (2019)

2.5. Potential for the application of plant extracts in the conservation of fish products

The trend toward the use of natural compounds is a viable alternative to alternatives to increase the shelf life of products that show accelerated deterioration reactions or are more susceptible to microbial contamination, such as fish products, since these agents tend to be less toxic than synthetic compounds, in addition to the possibility of full use of plants favoring the reduction of agri-food industry waste (Maqsood et al., 2012; Hassoun & Çoban, 2017). However, standardization in obtaining and applying these compounds is not as evident, which can be an obstacle to their wide use by the food industry since the beneficial responses of plant metabolites tend to have a distinct effect between groups of fish. From the data observed in the review, it was possible to observe that the application methods used in the shelf life tests of fish products were diverse (Table 2).

2.5.1. Methods of application of investigated plant compounds in shelf life tests

The most tested application methods among the 50 species studied in the 68 scientific papers were immersion (number of documents: 24), mixed (number of documents: 19), coating (number of documents: 11), immersion coating (number of documents: 7), ice coating (number of documents: 6) and salt coating (number of documents: 1). The application methods had a strong relationship with the test products; in the case of mixtures, the use was restricted to fish products such as sausages and fish burgers, while the ice coating was mainly used in tests with whole fish. Therefore, the studies tended to adapt the methods of application of active plant compounds to the conservation methods traditionally used.

In the study by Özogul et al. (2013), the extract of everlasting (*Helichrysum sp.*) and mistletoe (*Viscum album*) was obtained with 96% ethanol, while in the study by Ucak et al. (2018), the extraction of garlic (*Allium sp.*) was performed with 70% ethanol, both extracts were tested on fillets of rainbow trout (*Oncorhynchus mykiss*) at concentrations of 0.5% (w/v) and 5% (w/v). The maximum values of malondialdehyde after 14 days of refrigeration were 0.65 mg (MDA/kg^{-1}) and 4.41 mg (MDA/kg^{-1}), respectively. Despite the use of the same extraction and application methods, the study with the lowest concentration of applied extract had a more evident antioxidant effect. These results corroborate the need for further studies on the extraction method and evaluation of the relationship between the different compounds and possible benefits.

Studies that evaluated the immersion coating with compounds of the genus *Citrus sp.* in rainbow trout fillets (*Oncorhynchus mykiss*) obtained different responses to deteriorating chemical compounds after 12 days of refrigeration. In the study of the combination of 2% ethanol extract and vacuum packaging, the values of TBA and TVB-N were 0.52 mg (MDA/kg^{-1}) and 24.5 mg ($N/100g^{-1}$), while in the study with commercial EOs at 0.5% (w/v), the values were greater than 1.00 mg (MDA/kg^{-1}) and 30 mg ($N/100g^{-1}$), respectively (Mayeli et al., 2019; GhareAghaji et al., 2021).

Regarding the different responses between the application methods, studies that evaluated the application of ethanol extract of the genus Allium sp. in rainbow trout fillets (*Oncorhynchus mykiss*) showed different antioxidant activities. The study with immersion test in extract (5%) obtained a peroxide value of 7.33 ($meqO_2/kg^{-1}$) after 14 days of refrigeration, while in the study with application of dip coating enriched with extract (3%), the peroxide value was 20 ($meqO_2/kg^{-1}$) after 12 days of refrigeration (Raeisi et al., 2016; Ucak et al., 2018).

Significant results were also found in ice coating trials on marine species. The application of extracts of quinoa (*Chenopodium quinoa*) and brown algae (*Bifurcaria bifurcate*) added to ice showed effects in reducing lipid oxidation and controlling microbial growth in three species: European hake, Megrim, Atlantic chub mackerel (respectively, *Merluccius merluccius*, *Lepidorhombus whiffiagonis*, and *Scomber colias*) (Miranda et al., 2016; Miranda et al., 2018; Miranda et al., 2021). The use of these compounds can offer greater efficiency to the production chain since the main method of fish conservation by the fishing fleet is ice storage (Boeri et al., 1985). In addition to delaying the deterioration reactions after catching, the application of these extracts tends to reduce costs and losses in production, in addition to improving the hygienic sanitary quality of the fish products.

Among the analyzed studies, it was possible to observe that the concentrations of the extracts were heterogeneous, and the application description was not standardized. Different concentrations and descriptions are related to the structure of the compounds, which were used as EOs, liquid or solid extracts, films, and emulsions; therefore, the concentrations were described in volume, weight, or ppm. Standardization regarding the applied concentrations tends to propitiate an in-depth discussion and promote cocitations between the works. In addition, a detailed description of extract concentrations is essential, as the results are directly related to concentrations, and this assessment will be used in the investigation of the feasibility of using active plant compounds.

Different application concentrations conferred different effects; in assays with higher concentrations, the active potential tended to be higher. The shelf life of silver carp fillets (*Hypophthalmichthys molitrix*) was evaluated during refrigeration, and the fillets were immersed in ethanolic extracts of garlic (*Allium paradoxum*) and eryngo (*Eryngium caucasicum*) at concentrations of 2% and 4%, respectively. In general, the 2% concentrations give better results than the standard; however, the 4% tests extended the shelf life of the fillets by 1 week compared to the 2% test results for the chemical parameters of deterioration. Furthermore, overall acceptability values were higher for both extracts by 4% (mean overall acceptability: 7.75) compared to 2% (mean overall acceptability: 6.84) in sensory analysis after 6 days (Raeisi et al., 2017).

The shelf life tests focused mainly on the evaluation of fresh fish fillets; 50% of the scientific papers evaluated the application of active compounds in fillets, which is the main commercialization product for the fish sector. The effects of ethanolic, aqueous, lyophilized, and commercial EOs on prolonging the shelf life of fillets were significantly relevant.

The study by Apang et al. (2020) evaluated the reducing effect on the TVB-N value of ice-covered Indian mackerel fillets (*Rastrelliger kanagurta*) enriched with aqueous extracts of garcinias (*Garcinia indic*) and (*Garcinia cambogia*). According to Ariaii et al. (2015), silver carp fillets (*Hypophthalmichthys molitrix*) under refrigeration had amine values controlled by the action of the hydrodistilled extract of aniseed (*Pimpinella affinis*).

Effects on the control of lipid oxidation were described in studies with ethanolic extracts of pomegranate (*Punica granatum*) in fillets of narrow-barred Spanish mackerel (*Scomberomorus commerson*) (Khodanazary, 2019) and fillets of rainbow trout (*Oncorhynchus mykiss*) immersed in a solution with ethanolic extract of mistletoe (*Viscum album*) and hydrodistilled extract of zataria (*Zataria multiflora*) and clove (*Syzygium aromaticum*) submitted to refrigeration (Özogul, Kus & Kuley, 2013; Dehghani et al., 2018).

Some studies also carried out application tests on fish-based products. They are indicated as a mechanism for valuing the byproducts of the fish processing industry and tend to promote the consumption of fish, especially among consumers looking for products that are easy to prepare (Presenza et al., 2022). The application of active natural compounds in fish products can favor this approach, as they serve consumers who are looking for products with health aspects and extending the shelf life of food products makes them more viable. Ethanol and aqueous extracts of guabirá (*Campomanesia xanthocarpa*) and ginger (*Zingiber officinale*), respectively, indicate antimicrobial activity and delayed spoilage biochemical reactions, in addition to improving sensory attributes in tilapia fish burger (*Oreochromis niloticus*) (Mattje et al., 2019; Cristofel et al., 2021), and active extracts from leaves of *Achyroclines satureiodes* were added to sausages of tilapia. It delayed lipid oxidation, providing a prolonged shelf life under refrigeration tests (Lima et al., 2017).

Most of the studies carried out refrigerated shelf life tests, which correspond to an armament temperature close to 4 °C. Fish products are marketed refrigerated for fresh or frozen (-18 °C) products for fillet cuts or conventional products. Ninety-three percent of the studies carried out tests in refrigeration only, as, at higher temperatures, the spoilage metabolites are accelerated, reducing the time to analyze the effects. The study by Ali et al. (2019), with peel extracts of banana (*Musa sp*.), corroborates the facts that the peroxide values analyzed were 1.38 ($meqO_2/kg-1$) and 1.34 (meqO2/kg-1) for meatballs from carp (*Labeo rohita*) after 9 days of refrigeration and 60 days of freezing, respectively.

2.5.2. Investigated benefits of the application of plant compounds in fish products

The response variables analyzed in the shelf life tests focused on the aspects of lipid oxidation and microbial contamination, which are the main critical factors in the deterioration of fish products (Nie et al., 2022).

Some groups of fish are rich in polyunsaturated fatty acids (PUFAs) that are directly related to nutritional benefits, such as the reduced risk of chronic noncommunicable diseases (NCDs) (Chen et al., 2022). However, this lipid composition is highly susceptible to oxidation resulting from a series of autolytic reactions by hydroperoxidation. In the evaluation of lipid oxidation, scientific papers have mainly investigated the effects on the formation of malondialdehyde (MDA), an oxidation biomarker formed from chemical reactions of secondary lipid oxidation. MDA has been investigated as a substance with potential health risks since it has an active effect on physiological process factors (Reitznerová et al., 2017); therefore, limits on the presence of MDA in fish can help ensure consumer safety. Although the critical limits for the presence of MDA in fish are not evident, lipid oxidation causes fish to become rancid, generating sensory rejection. Studies still point to a problem regarding the analysis of MDA. Most of the scientific papers analyzed carried out a quantitative assessment of MDA through the spectrophotometry method of 2-thiobarbituric acid; however, this methodology has low stability and repeatability, and the values tend to be overestimated when compared to more stable methods such as HPLC (Mendes et al., 2009; Reitznerová et al. 2017).

Most plant secondary compounds have effects on lipid oxidation. However, the mechanisms of action are varied, mainly in the scavenging or neutralization of free radicals (Maqsood et al., 2012). Among the 68 scientific papers, 42 presented some answers on the prevention of lipid oxidation. MDA values were lower at all concentrations of pistacia (*Pistacia sp.*) (Hassanzadazar et al., 2021) in a test with minced rainbow trout (*Oncorhynchus mykiss*) after 12 days of refrigeration compared to the control. This result was also evidenced in Asian sea bass fillets (*Lates calcarifer*) immersed in concentrations higher than 1429 mg/L of *Cinnamon sp*. extract (Chuesiang et al., 2020).

Another marker of self-deterioration is total volatile basic nitrogen (TVB-N), metabolites resulting from the degradation of proteins by the accumulation of organic amines; these compounds are toxic and affect relevant characteristics in fish acceptance (Bekhit et al., 2021). According to the European Commission, the maximum acceptable limits of TVB-N in fish muscle range from 25 to 35 mg (N/100 g^{-1}) between species (EC, 2008).

Apang et al. (2020) studied the application of garcinia (*Garcinia cambogia*) peel extract on ice and extended the shelf life of Indian mackerel fillets (*Rastrelliger kanagura*) by 1 week, considering the critical limits of TVB-N. Active extracts of seaweeds (*Spirulina platensis* and *Chlorella vulgaris*) also showed a reduction in TVB-N values when compared to the control, and the shelf life extension in the tests with refrigerated sardine fillets (*Sardinella aurita*) was greater than 5 days compared to the control (Özogul et al., 2021).

Antimicrobial effects were also evaluated, and pathogenic microorganisms have a high ability to proliferate in fish products, which can cause relevant outbreaks of foodborne illness. In addition, spoilage bacteria tend to increase histamine levels in fish muscles (Sheng & Wang, 2020). In this context, intervention strategies on microbiological quality are extremely relevant, both for the fishing industry and for aquaculture, which is very concerned with microbiological resistance in production and postharvest. Studies indicated significant responses in microbial control in ice-chilled specimens of European hake (*Merluccius merluccius*) enriched with aqueous extract of the macroalgae *Bifurcaria bifurcata*, and the values of psychotropic bacteria were lower after 13 days of storage compared to tests with traditional ice (Miranda et al., 2021).

2.5.3. The shelf life of the entire production chain of fish products

From the set of scientific papers investigated, it was possible to observe that the shelf life tests were evaluated only in ichthyic species, with 48.53% of marine species and 47.06% of freshwater fish, and another 4% were not identified in the article. Rainbow trout (*Oncorhynchus mykiss*) was the most investigated species among the studies (number of documents: 17). According to FAO (2020), this species ranks 15th in total world aquaculture production, with 848.1 thousand tons produced in 2018. Other freshwater fish species were also evaluated, such as specimens of the genus *Oreochromis sp*. and the species silver carp (*Hypophthalmichthys molitrix*).

The marine group showed greater dispersion among the investigated species; among the 33 studies that used marine species, 25 were distinct. Species of the genus *Scomber sp*., followed by the species Atlantic salmon (*Salmo salar*) and European pilchard (*Sardina pilchardus*), present greater recurrence among marine species in the analyzed studies.

The higher rate of use of marine species to investigate the effect of active compounds in plants to prevent spoilage activities may be related to the intrinsic composition of these species, as their diet is rich in foods that provide them with a large amount of polyunsaturated fatty acids (PUFAs), which are still more susceptible to deteriorating activities such as lipid oxidation. In addition, the structure of the extractive fishing chain is more fragile to establish quality control standards when compared to aquaculture products. In turn, the species from aquaculture investigated among the analyzed scientific works showed homogeneity, which is due to the standardization of production processes, mainly in the valorization of species that present good levels of production and consumer acceptance.

Furthermore, despite the importance in terms of production, economy, trade, and culture, none of the studies analyzed in this review presented data on tests of the application of plant bioactive compounds in other groups of aquatic organisms, such as mollusks and crustaceans, which are also highly susceptible to degradation.

2.6. Conclusion and Future Prospectus

The review allowed the analysis of scientific articles that evaluated the application of plant compounds such as extracts and essential oils with bioactive potential to prolong the shelf life and delay the deterioration of fish products. Research on active plant compounds has attracted the attention of many researchers for several years, and application tests of these compounds in fish products indicate a synergistic trend between health aspects and consumer acceptance.

From the bibliometric analysis, it was possible to verify that the subject has already been studied in at least 24 countries, and although some species of plants and fish are recurrent in the studies, the number of coauthorships is low, indicating the possibility that, in the future, international cooperation will improve the results.

The review allowed us to identify a trend in the use of organic solvents as a satisfactory method of extracting phenolic compounds; however, more detailed investigations on the composition of extracts and EOs can corroborate more promising data regarding commercial viability, since the compounds tend to have different effects on the shelf life of fish products. Regarding the methods of application of plant bioactive compounds, it was possible to verify the potential of their use in various forms of application, with most scientific articles showing significant effects on life extension both in tests with marine and freshwater species, mainly in delaying lipid oxidation, proving to be a viable alternative for promoting aspects of healthiness, food safety, and cooperation for more sustainable food systems.

From this review, it was possible to identify some limitations that need to be better investigated in future studies. In addition, the grouping and crossing of data and visual maps can help in future research on the subject, and studies on the application of plant extracts to prolong shelf life can be further explored in other groups, such as crustaceans and mollusks, which also have a high susceptibility to spoilage and considerable commercial importance.

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3. SIMPLEX-CENTROID MIXTURE DESIGN AS A TOOL TO EVALUATE THE EFFECT OF ADDED FLOURS FOR OPTIMIZING THE FORMULATION OF NATIVE BRAZILIAN FRESHWATER FISH BURGER

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Abstract

The development of fish-based products can meet consumers' desires related to sensory, nutritional, and convenience aspects. In addition, it can contribute to the waste reduction of undervalued fish portions. This study presents the results of the optimization of fish burger formulations based on mechanically separated meat (MSM) of tambaqui (*Colossoma macropomum*) with oatmeal and cassava starch, using a simplex-centroid mixture design (SCMD) with total flour concentrations ranging from 0% to 10%, and MSM from 82.5% to 92.5%. The formulations were evaluated for physicochemical, yield, and sensory characteristics. The addition of flour had a positive effect ($p \leq 0.05$) on physicochemical and sensory properties, resulting in a 12% higher cooking yield compared to the formulation without flour. All formulations scored high acceptance; however, the formulation with the binary combination between flours showed the best ranking in the preference test and presented a score of 7.58 on the 9-Point hedonic Scale of global acceptance. Hardness was higher in the pure pseudo-component combinations but provided a reduction in binary and ternary combinations. The study shows the importance of optimization tools in product development aiming to consider yield and cost aspects.

Keywords: Fish products, Sensory analysis, Optimization design, Formulation parameters.

3.1. Introduction

The nutritional importance of fish and its performance in reducing cardiovascular disease and its positive effects on the anti-inflammatory and cognitive systems are known (Chen, Jayachandran, Bai & Xu, 2022; Krittanawong et al., 2021; Martí & Fortique, 2019; Mohanty et al., 2019). Notwithstanding the recommendations for consumption, there is concern about the origin of fish, as some marine stocks are potentially depleted (WHO, 2003). Thus, approaches to increasing consumption based on aquaculture products are gaining momentum, as aquaculture techniques are seen as a window of opportunity for sustainable expansion (Gephart et al., 2021; Nature, 2021).

The study by Naylor et al. (2021) corroborates this context, indicating that the inclusion of sustainably managed fish in the human diet will depend on the species exploited and on production practices. Aquaculture has been developing exponentially with increasingly advanced systems, representing a solution for a continued supply of fish (FAO, 2020). Brazilian aquaculture follows this trend. In 2020 aquaculture production reached a record with an annual growth of 5.93% (Brazilian Association of Fish Farming, 2021). Nevertheless, productive challenges do persist, mainly related to two aspects: environmental risks, due to the impacts on the reduction of native ichthyofauna through the negligent control of exotic species (Fragoso-Moura, Porto, Maia-Barbosa, & Barbosa, 2016; Latini & Petrere, 2004; Pelicice, Vitule, Lima Jr., Orsi & Agostinho, 2013); and the nutritional performance of each species (Berntssen et al., 2021; Henderson & Tocher, 1987).

In this context, native species with suitable productive performance tend to stand out, especially the tambaqui (*Colossoma macropomum*, Cuvier 1818), a native species of the Amazon River basin, currently among the most commercialized and widely spread native species in Brazilian fish farming, with remarkable productive and commercial records (Brazilian Association of Fish Farming, 2021). The production technology packages for the species are adapted to different cultivation systems, stocking densities, and varied feeding (Merola & Cantelmo, 1987; Reis et al., 2019; Santos et al., 2021).

Although there is a remarkable overview in terms of production, the consumption of fish from aquaculture is restricted by the choice profile of consumers, who still choose species from extractive capture as they believe they are related to safety and trustworthiness (López-Mas et al., 2021; Mitra, Khatun, Prodhan & Khan, 2021). A study carried out in different regions of Brazil showed a lack of consumer knowledge about the origin of fish; most consumers accepted Nile tilapia (*Oreochromis niloticus)* but rejected fish from aquaculture. Considering that the supply of Nile tilapia (*Oreochromis niloticus*) in Brazil is exclusive from aquaculture, there is a need to define strategies for cultural change in fish consumption (Pedroza Filho et al., 2020). Another study that evaluated the sensory acceptance of wild and cultivated tambaqui (*Colossoma macropomum*) indicated a difference in the organoleptic aspects, where cultivated fish has shown greater acceptance, probably due to the quality of possible management of aquaculture systems (Sousa et al., 2020).

Also, in the context of consumption, it is necessary to consider that fish is sold entirely or in traditional cuts, such as fillets, and processes that attempt to add value or use all parts of the fish are rare, resulting in the underutilization of food by the fish industry (FAO, 2020). An alternative at this point is the new food product development (NFPD), which can provide safe and nutritious food with the minimal addition of additives and based on renewable resources (Azanedo, Garcia-Garcia, Stone & Rahimifard, 2020; Garcia-Garcia, Azanedo & Rahimifard, 2021). Convenience food products are a possible strategy, being increasingly present in the choice of consumers who, in addition to convenience, value sensory and nutritional characteristics (Contini, Boncinelli, Gerini, Scozzafava & Casini, 2018; Darian & Cohen, 1995). Although studies on fish-based convenience foods such as tambaqui (*Colossoma macropomum*) fish burgers indicate sensory acceptance (Anjos et al., 2021; Lima et al., 2020) there is still a need for research to improve the development process, focusing on commercial and healthiness aspects (Duran et al., 2017). Process approaches, such as cost reduction and fiber addition by applying flours and starches to fish burgers, have been discussed in the study by Dilucia, Lacivita, Del Nobile & Conte (2021). However, the effects of these ingredients were not evidenced. In another more detailed approach, the addition of flaxseed flour had a significant effect on moisture retention and cooking yield, and additions greater than 10% tended to lower consumer acceptance (Duman, 2020).

Applied statistical methods, such as optimization models, contribute to the formulation of new products, suitable for commercial scale, considering consumers' demands and preferences. Simplex-centroid mixture design (SCMD) results allow for to identify of the synergistic effects of mixtures and predict models that provide answers, such as high quality and low costs (Calado & Montgomery, 2003; Cornell, 2011). The objective of this work was to develop a fish burger based on mechanically separated meat (MSM) obtained from tambaqui (*Colossoma macropomum*) to promote the consumption of Brazilian native fish from aquaculture production, with the addition of flour as a functional ingredient, using a simple design with three variables (MSM, oatmeal and cassava starch) to optimize a new clean label product formulation.

3.2. Material and methods

3.2.1. Raw materials and MSM preparation

Twelve samples (N = 12) consisting of tambaqui (*Colossoma macropomum*) bands were supplied by a local producer (Piracicaba/SP, Brazil). The samples, from the same batch and produced in an aquaculture system, were received frozen by the Fish Processing Laboratory ESALQ/USP (Piracicaba/SP, Brazil) and had an average weight of 1.2 kg. Then the bands were de-frosted, and the head and skin were removed before obtaining mechanically separated meat in a mincer (High Tech 250C). The mechanically separated meat (MSM) was homogenized and packed into 1 kg packages, frozen at -18 °C. Other ingredients: oatmeal; cassava starch; NaCl; monosodium glutamate, and spices, were purchased at the local market. The yields concerning the band and the MSM mass were registered and calculated according to the following equation.

$$\%Skin\ yield = \frac{skin\ weight}{fish\ band\ weight}\ x\ 100 \tag{1}$$

$$\%Head\ yield = \frac{head\ weight}{fish\ band\ weight}\ x\ 100 \tag{2}$$

$$\%MSM\ yield = \frac{MSM\ weight}{fish\ band\ weight}\ x\ 100 \tag{3}$$

3.2.2. Formulation development of fish burgers using simplex-centroid mixture design

To optimize the fish burger formulation, the simplex-centroid mixture design (SCMD) method was applied using the Statistica® 13.3 software from TIBCO (Palo Alto, California, USA). The adjustment for the simplex region established for the study was delimited into three pseudo-components (independent variables (X1: MSM; X2: oatmeal; X3: cassava starch)), ranging from 0 to 1. The SCMD consisted of 7 different formulations, to avoid systematic errors, the experiments were performed randomly. Points 1, 2, and 3 (triangular vertices) correspond to the pure pseudo-components. Points 4, 5, and 6 were the binary mixtures of two, and point 7 (the center of the triangle) was the ternary mixture. The formulations are described as F1:0C0, F2:O10, F3:C10, F4:O5, F5:C5, F6:O5C5, and F7:O3C3, respectively.

The design points in the Simplex-Centroid are represented in triangular diagrams by polynomial equations that will be defined by the qth-order mixture. In the case of ternary mixtures where q = 3 the special cubic model (Eq. (4)) must be used, allowing the prediction of the values of response surfaces.

$$\hat{y} = \sum_{i=1}^{q} \beta_i x_i + \sum\sum_{i<j}^{q} \beta_{ij} x_i x_j + \sum\sum\sum_{i<j<k}^{q} \beta_{ijk} x_i x_j x_k + \epsilon \quad (4)$$

Interactions are evaluated in b_i, b_{ij} and b_{ijk}; x_i, x_j and x_k are the studied factors and $\hat{y}$ is the experimental response to the factors.

Subsequently, the SCMD coded experimental points were configured in the original components (Table 1), the minimum and maximum levels of the independent variables, and the other ingredients were fixed in the formulations: NaCl (1.5%); monosodium glutamate (0.2%), white pepper (0.1%), garlic powder (0.3%), onion powder (0.4%) and ice (5%), were based on previous studies (Delfino et al., 2021; Oliveira et al., 2020) and pre-tests in the laboratory.

Table 1. Simplex-centroid mixture design applied to optimize fish burgers formulation.

Run	Pseudo-components			Original Components (%)		
	X_1	X_2	X_3	MSM (X_1)	Oatmeal (X_2)	Cassava Starch (X_3)
1	1	0	0	92.5	0	0
2	0	1	0	82.5	10	0
3	0	0	1	82.5	0	10
4	0.5	0.5	0	87.5	5	0
5	0.5	0	0.5	87.5	0	5
6	0	0.5	0.5	82.5	5	5
7	0.333	0.333	0.333	85.84	3.33	3.33

Afterward, the formulations were weighed, and the ingredients were homogenized. Portions of 90g were molded into suitable formats of 10 cm in diameter, placed in low-density polyethylene bags, and stored at -18 °C until processing and analysis.

3.2.3. Physicochemical and microbiological analysis of MSM and fish burgers

3.2.3.1. Microbiological analyses

To determine microbiological safety, 25 g of MSM and each formulation were analyzed in triplicate. Samples were homogenized (ITR MC-1204) with 225 mL of 0.1% peptone salt solution for enumeration of total coliforms, *Escherichia coli*, and coagulase-positive staphylococci, another 25 g sample was homogenized in 225 mL of 1% buffered peptone solution (pre-enrichment) for analysis of *Salmonella spp*.
The analyses of total coliforms and *Escherichia coli* were carried out following the AOAC Method 998.08:2002 (3M Petrifilm), coagulase-positive staphylococci according to ISO 6888-2:2021, both results were expressed in CFU/g−1, and *Salmonella spp*. according to ISO 6579:2017, expressed as absence or presence per 25 g.

3.2.3.2. Proximate composition

For analysis of the proximate composition of MSM and fish burger formulations, the tests were performed on raw samples by AOAC official methods (2005). Crude protein was determined by the Kjeldahl method (using N x 6.25 as a conversion factor); the fat was extracted with hexane in a Soxhlet type extractor; moisture was determined by the gravimetric method in an oven with air circulation at 105 °C; the ash content was determined by incineration in a muffle at 550 °C. The carbohydrate level of the fish burgers was determined by subtracting the moisture, protein, fat, and ash percentages from 100. The energy value was determined based on 100 g, by multiplying the crude protein and carbohydrates by 4 and fat by 9.

3.2.3.3. Water activity (a_w) and pH measurement

Water activity (a_w) was determined using a standard hygrometer (AquaLab 4 TE, Decagon Devices, Pullman, USA). The pH was measured on the raw fish burger using a pH meter (Tecnal Tec-3MP, Piracicaba, Sao Paulo, Brazil). Measurements were performed in triplicate.

3.2.4. Fish burger evaluation analysis

The fish-burger samples were cooked on an electric plate at 150 °C for 4 min on each side until the internal temperature reached 75 °C, and then samples were cooled to room temperature (the same process was used in preparing samples for sensory analysis).

3.2.4.1. Instrumental color measurement

Color measurement of fish burgers was determined using a colorimeter (Chroma Meter CR-400, Konica Minolta, Japan), measuring an area of 8 mm in diameter. *L** (luminance ranging from 0 (black) to 100 (white)), color coordinates *a** (green to red), and *b** (blue to yellow) were examined. The analysis was performed in triplicate on each side of the fish burger, using three of each formulation, totaling 18 readings per treatment.

3.2.4.2. Texture profile analysis (TPA)

Texture profile analysis (TPA) was performed using a Texture Analyzer (Stable Micro Systems, TA-XT-PLUS, Godalming, Surrey, UK). Cylindrical samples were cut from fish burgers, with a 17 mm diameter and a height of 10 mm, and subjected to a two-cycle compression test. Samples were compressed to 50% of their original height with a cylindrical aluminum probe (P/35 35 mm diameter), with a test speed of 1 mm s^{-1} and a post-test speed of 10 mm s^{-1}. The analysis was performed using three fish burgers of each formulation, totaling nine values per treatment. The parameters determined were hardness, cohesiveness, springiness, and chewiness, as described by Bourne, Kenny, and Barnard (1978).

3.2.4.3. Technological cooking properties

The weight of the fish burgers was measured using an analytical scale and a digital caliper before and after the cooking process on three fish burgers of each formulation. Cooking yield, diameter reduction, thickness reduction, moisture retention, and fat retention were determined as described by Sánchez-Zapata et al. (2010) measured according to the following equations:

$$\%Cooking\ yield = \frac{cooked\ weight}{raw\ weight}\ x\ 100 \quad (5)$$

$$\%Diameter\ reduction = \frac{raw\ fish\ burger\ diameter - cooked\ fish\ burger\ diameter}{Raw\ fish\ burger\ diameter}\ x\ 100 \quad (6)$$

$$\%Thickness\ reduction = \frac{raw\ fish\ burger\ thickness - cooked\ fish\ burger\ thickness}{raw\ fish\ burger\ thickness}\ x\ 100 \qquad (7)$$

$$\%Moisture\ retention = \frac{cooked\ weight\ x\ \%moisture\ in\ cooked\ fish\ burger}{raw\ weight\ x\ \%moisture\ in\ raw\ fish\ burger}\ x\ 100 \qquad (8)$$

$$\%Fat\ retention = \frac{cooked\ weight\ x\ \%fat\ in\ cooked\ fish\ burger}{raw\ weight\ x\ \%fat\ in\ raw\ fish\ burger}\ x\ 100 \qquad (9)$$

Moisture, fat (Soxhlet), and protein (Kjeldahl) of cooked fish burgers from Equations (7), and (8) were determined using AOAC (2005) methods.

3.2.5. Quantitative descriptive analysis

The quantitative descriptive analysis (QDA) was performed based on the recommendations of Stone & Sidel (2004). All procedures were approved by the Committee for Ethics in Research on Human Being (CEP ESALQ-USP) under number CAAE: 43321621.7.0000.5395. Candidates were recruited among students of the Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ-USP) through an online form. Nineteen candidates, out of the twenty-seven recruited, were pre-selected through a basic taste recognition test (no errors for 6 solutions) and an odor recognition test (a minimum of 8 correct answers out of a total of 10 solutions) according to ISO 3972:2011 and ISO 8586:2012.

3.2.5.1. Development of descriptive terminology

The sensory attributes were raised by the nineteen panelists based on a study by Quadros, Rocha, Ferreira & Bolini (2015). After discussions to reach a consensus, the descriptive terms that were most important to characterize the appearance, aroma, taste, and texture were selected. The sensory panel, in consensus with the assistance of a leader, also defined the minimum and maximum intensity references for each attribute (Table 2).

Table 2. Attributes, definitions, and reference samples developed by the sensory panel for MSM-based fish burger obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Attribute	**Descriptor**	**Definition**	**Reference**
Appearance	Grilled color	Caramelized color intensity	Weak: fish burger baked in aluminum foil Strong: grilled fish burger
	Thickness	Visual perception of the characteristic thickness	Weak: fish burger with 0.5 cm thickness Strong: fish burger with 1.2 cm thickness
	Compaction	Perception of the completeness of the fish burger	Weak: minced meat fish burger Strong: fish burger based on MSM
Aroma	Fish odor	Intensity of characteristic fish odor	Weak: grilled fish burger Strong: fish burger cooked in water
	Rancid odor	Intensity of characteristic rancid odor	Weak: grilled fish burger Strong: fish burger dipped in soy oil
Flavor	Fish flavor	Perception of the amount of characteristic fish taste	Weak: grilled fish burger Strong: fish burger cooked in water
	Seasoned	Perception of the amount of seasoning and spices	Weak: grilled fish burger Strong: fish burger dipped in soy oil
	Saltiness	Perception of the amount of salt	Weak: fish burger control with low addition of salt and condiments Strong: grilled fish burger
	Greasy	Perception of the amount of fat	Weak: fish burger control with low addition of salt and condiments Strong: grilled fish burger
Texture	Hardness	Force required to deform the fish burger	Weak: fish burger grilled in 70 °C Strong: fish burger grilled in 150 °C
	Succulence	Perception of the amount of liquid released from the fish burger in the mouth	Weak: fish burger grilled in 150 °C Strong: fish burger grilled in 70 °C
	Crust	Perception of the crunchiness of the crust when chewing the fish burger	Weak: fish burger grilled in 70 °C Strong: fish burger grilled in 150 °C

3.2.5.1. Training and selection

Training for sensory memory formation and leveling was carried out in three sessions for each panelist, through direct contact with the maximum and minimum intensity references for each attribute. After the training stage, the panelists were selected according to their discriminatory power, repeatability, and consensus with the panel, according to ISO 8586:2012. Twelve out of nineteen were selected to perform analysis on the sensory profile of fish burgers. The final panel was composed of 60% women and 40% men, aged between 22 and 43 years.

3.2.5.2. Final assessment

Analysis was performed at the Sensory Analysis Laboratory of the Department of Agri-food Industry, Food and Nutrition (ESALQ-USP) in individual cabins (22 °C), under white light, according to ISO 8589:2007 and ASTM International (2017). The seven fish burger formulations were presented to the selected panelists on plates encoded with random three-digit numbers. The fish burgers were presented randomly and evaluated on an unstructured linear intensity scale of 90 mm in length for each attribute, using vocabulary developed by the trained panel, they were instructed to use water and a cream cracker to clean the palate between samples.

3.2.6. Sensory affective testing

The affective test was carried out with 12 trained panelists to verify the global acceptance of the fish burger as described by Stone and Sidel (2004). The test consisted of a 9-point hedonic scale with verbal anchors (1 dislike extremely and 9 like extremely), purchase intention (1 definitely will not buy and 5 definitely will buy), and a preference ranking test. The seven fish burger formulations were presented to trained panelists on plates encoded with random three-digit numbers.

3.2.7. Statistical analysis

All determinations were run at least three times. The values of different parameters were expressed as the mean standard deviation, using analysis of variance (ANOVA) and then Tukey's test was applied to compare the means with a significance level of

5%. Data were analyzed using the Statistica® 13.3 software from TIBCO (Palo Alto, California, USA).

3.3. Results and discussion

3.3.1. Physicochemical analysis and microbiological analysis

The yield of mechanically separated meat (MSM) of tambaqui (*Colossoma macropomum*) was 59.81% compared to the weight of the entire band. Considering the disposal of the head (24.92%) and skin (9.38%), the yield was 91.04%. Similar results were found in the MSM yield evaluation of catfish (*Clarias gariepinus*), with 57.33% and 93.88% in relation to living weight and clean trunk, respectively (Daga et al., 2020). The moisture content of the MSM was 71.89 ± 0.24%, 10.05 ± 0.17% fat, 15.78 ± 0.55% protein, 1.28 ± 0.04%, and 1.01 ± 0.26% ash and carbohydrate, respectively. Water activity was 0.99 and pH 6.53. Similar values were observed for the MSM of red porgy (*Pagrus pagrus*) (Guimarães et al., 2018), and MSM of catfish (*Brachyplatystoma vaillantii*) (Oliveira, Lourenço, Sousa, Peixoto Joele & Ribeiro, 2015).

The results of the proximate composition of fish burger formulations described in Table 3, showed significant differences in moisture, ash, fat, and protein contents ($p \leq 0.05$). As the raw material and ingredients used were the same, the variation in the final composition of the formulations was due to the interaction between the pseudo-components. However, the addition of flours had no significant effect ($p \geq 0.05$) on a_w and pH, the results varied between 0.97 and 0.98, 6.40 and 6.47, respectively.

The main proximate constituent being moisture, it is also the component that suffers the most variation, mainly due to its interaction with fat, corroborating the most notable effect of the addition of flour on moisture and lipid composition. A study that evaluated the addition of cassava starch in the development of fish burgers also showed a reduction in moisture (Pires et al., 2017). Another study that evaluated the addition of oatmeal showed a reduction in moisture, and reduction in lipids in the formulation of fish burgers with Nile tilapia (*Oreochromis niloticus*) pulp (Braga, Pasquetti, Bueno & Merengoni, 2008). However, the formulation with the highest concentration of oatmeal (F2:O10) had an additive effect on lipid concentration. A similar result was observed for the lipid composition in addition to more than 10% flaxseed flour in the fish burger (Duman, 2020).

The results on the proximate composition of the formulations showed values correlated with studies without the addition of fat substitutes, as in the study with fish burgers of silver catfish (*Rhamdia quelen*) filleting residue, which presented values of 67.89%, 2.97%, 7.24% and 16.74% for moisture, protein, lipid, ash, and carbohydrate content, respectively, when the addition of residues was greater (Bochi, Weber, Ribeiro, Victório & Emanuelli, 2008). Anjos et al. (2020) found correlated values for moisture in tambaqui fish burgers (*Colossoma macropomum*), enriched with green banana and chitosan biomass. The flour concentrations used by the experimental design did not have a significant effect on the energetic value when compared to the formulation without flour.

To ensure the hygienic-sanitary quality of the study, the MSM and fish burger formulations were analyzed and presented following the established criteria by Normative Instruction No. 60 of the National Health Surveillance Agency (ANVISA) (BRAZIL, 2019). The results indicated the absence of *Salmonella spp*. (in 25g) and *Escherichia coli* (CFU/g) in all samples, and the samples of MSM, F2:O10, F4:O5, F5:C5, and F7:O3C3 presented values ≤ 1.0 × 101 for coagulase-positive staphylococci (CFU/g) and were absent in the other formulations. Microbiological patterns of the fish burgers were also evidenced accordingly in the studies by Fogaça, Otani, Portella, Santos-Filho & Sant'Ana (2015), in the microbiological evaluation of fish burgers with MSM of Nile tilapia (*Oreochromis niloticus*), and in the evaluation of the effects of grape pomace flour on the quality parameters of salmon burgers (*Salmo salar*) (Cilli et al., 2019).

3.3.2. Texture profile

The use of trans fats in conventional products has been increasingly questioned by consumers. Conversely, it has a strong effect on the texture of molded animal foods (Hygreeva, Pandeia & Radhakrishna, 2014). However, studies show that replacing functional ingredients like fibers and starches improves the stability of animal products (Schmiele, Mascarenhas, Barretto & Pollonio, 2015; Talukder, 2015).

The results of the effect of adding flour on the fish burger texture are shown in Table 4. The hardness that describes the force required for deformation of the fish burger between the molar teeth showed similar values in the study with a fish burger of Nile tilapia (*Oreochromis niloticus*) (Bainy, Bertan, Corazza & Lenzi, 2015).

According to Gao, Zhang & Zhou (2014), there is an inversely proportional relationship between moisture and hardness, which explains the lower textural values of the formulation without the addition of flour (F1:O0C0) While cassava starch affected textural characteristics, these effects were not visible on the response surface (Fig. 1).

The addition of wheat bran in biquara fish burgers (*Haemulon plumierii*) showed a reducing effect on hardness and cohesiveness (Raúl et al., 2018). However, the results of the addition of oatmeal and cassava starch in higher concentrations indicated an increase in hardness and cohesiveness, possibly because these ingredients are fibrous and tend to weaken myofibrillar bonds. It is necessary to consider that the addition of flour favors the retention of macronutrients and tends to improve the integrity of the product.

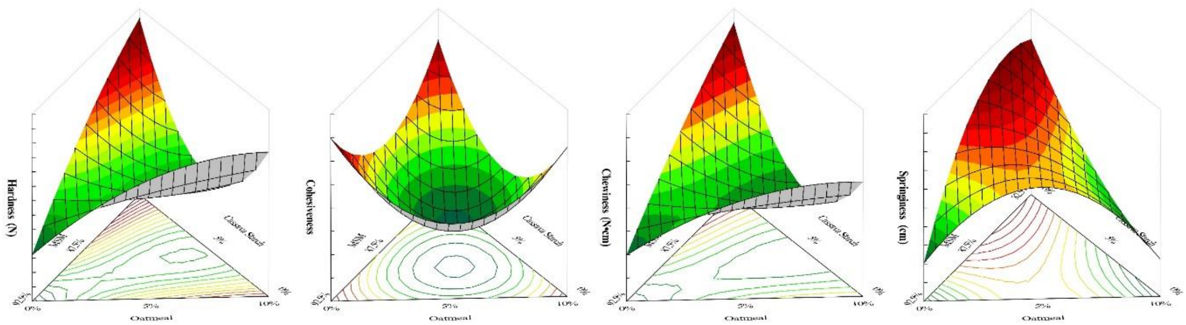

Figure 1. Response surface plots for effects of adding oatmeal and cassava starch on the instrumental texture of fish burger formulations.

A previous study on the effects of starch properties on the textural characteristics of fish burgers, using surimi (*Merluccius hubsi*), showed similar results for cohesiveness and springiness, but hardness and chewiness values were lower (Coelho, Weschenfelder, Meinert, Amboni & Beirão, 2007). Compared to beef burgers, the values of cohesiveness, springiness, and chewiness were also similar (Carvalho, Milani, Trinca, Nagai & Barretro, 2017; Trevisan, Bis, Henck & Barretto, 2016).

Table 3. Physicochemical composition of MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Form.	Pseudo-components			Responses							
	X_1	X_2	X_3	Moisture	Ash	Fat	Protein	Carbohydrate	Kcal	a_W	pH
F1:O0C0	1	0	0	71.55 ± 0.31[a]	2.67 ± 0.00[ab]	8.82 ± 0.03[b]	15.26 ± 0.63[ab]	1.69	166.23	0.97[a]	6.46[a]
F2:O10	0	1	0	62.87 ± 0.40[e]	2.83 ± 0.05[a]	9.29 ± 0.02[a]	16.21 ± 0.34[a]	8.80	164.41	0.98[a]	6.40[a]
F3:C10	0	0	1	65.25 ± 0.37[d]	2.61 ± 0.04[b]	7.96 ± 0.04[e]	14.37 ± 0.03[b]	9.81	162.95	0.97[a]	6.46[a]
F4:O5	0.5	0.5	0	68.51 ± 0.47[bc]	2.73 ± 0.10[ab]	8.59 ± 0.12[c]	15.85 ± 0.17[a]	4.33	164.02	0.98[a]	6.43[a]
F5:C5	0.5	0	0.5	67.77 ± 0.07[c]	2.72 ± 0.01[ab]	8.82 ± 0.07[b]	15.45 ± 0.06[ab]	5.25	159.63	0.98[a]	6.46[a]
F6:O5C5	0	0.5	0.5	65.68 ± 0.20[d]	2.41 ± 0.02[c]	8.31 ± 0.05[d]	15.09 ± 0.27[ab]	8.51	168.41	0.98[a]	6.44[a]
F7:O3C3	0.333	0.333	0.333	67.56 ± 0.24[c]	2.55 ± 0.03[bc]	7.99 ± 0.02[e]	15.08 ± 0.27[ab]	6.82	162.42	0.98[a]	6.47[a]

Results are means ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

Table 4. Instrumental texture and color parameters (*L**, *a** and *b**) of the formulations MSM-based fish burger obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Form.	Pseudo-components			Responses						
	X_1	X_2	X_3	Hardness (N)	Cohesiveness	Springiness (cm)	Chewiness (N·cm)	*L**	*a**	*b**
F1:O0C0	1	0	0	20.46 ± 2.54[b]	0.59 ± 0.04[a]	0.66 ± 0.00[c]	7.95 ± 0.49[b]	56.04 ± 3.24[a]	5.34 ± 1.07[a]	18.94 ± 2.81[a]
F2:O10	0	1	0	34.09 ± 4.44[a]	0.61 ± 0.02[a]	0.66 ± 0.03[c]	13.82 ± 2.46[a]	51.97 ± 2.20[a]	7.13 ± 1.71[a]	24.88 ± 3.00[a]
F3:C10	0	0	1	38.67 ± 1.89[a]	0.61 ± 0.03[a]	0.79 ± 0.00[a]	18.80 ± 1.74[a]	54.59 ± 2.67[a]	5.35 ± 1.34[a]	21.25 ± 4.33[a]
F4:O5	0.5	0.5	0	30.61 ± 2.78[a]	0.54 ± 0.01[a]	0.74 ± 0.02[ab]	12.49 ± 1.62[a]	52.21 ± 2.45[a]	7.38 ± 2.05[a]	24.64 ± 3.74[a]
F5:C5	0.5	0	0.5	31.30 ± 6.93[a]	0.57 ± 0.03[a]	0.78 ± 0.01[a]	14.00 ± 3.95[a]	51.25 ± 2.25[a]	7.17 ± 1.79[a]	24.16 ± 3.73[a]
F6:O5C5	0	0.5	0.5	24.21 ± 2.23[ab]	0.55 ± 0.01[a]	0.70 ± 0.01[bc]	9.39 ± 0.93[ab]	54.50 ± 2.61[a]	5.93 ± 1.84[a]	22.94 ± 3.83[a]
F7:O3C3	0.333	0.333	0.333	24.19 ± 1.37[ab]	0.52 ± 0.04[a]	0.74 ± 0.03[ab]	9.64 ± 0.88[ab]	50.01 ± 1.94[a]	8.93 ± 1.70[a]	27.83 ± 3.51[a]

Results are means ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

3.3.3. Color instrumental measurements

The results of the color parameters of the cooked fish burger formulations are shown in Table 4. Color assessment is an important tool for food products as it directly affects the sensory acceptance of consumers (Clydesdale, 1991) mainly for products that use cooking processes, such as fish burgers, which undergo various reactions such as the Maillard effect, which, in addition to adding a caramelized appearance, can mask undesirable colors (Ames, 1992; Starowicz & Zieliński, 2019). Similar results for *L**, *a**, and *b** values were found for the blended fish burger with different percentages of shrimp, camel, and ostrich (Shekarabi, Monjezi, Shaviklo & Mohamed, 2020).

Other studies that used white meat fish also presented correlated values, such as the study by Raúl et al. (2018) with the addition of different wheat bran in fish burgers of biquara (*Haemulon plumierii*), and fish burgers produced with different levels of filleting catfish (*Rhamdia quelen*) (Bochi et al., 2008). According to Cilli et al. (2019) in the evaluation of the effects of grape pomace flour on quality parameters of salmon burgers (*Salmo salar*), observed higher values for *L** and *a** and lower values for *b**. The addition of different ingredients tends to modify the food color. The different flour concentrations indicated a decrease in the *L** band and an increase in the *a** and *b** bands. However, the changes were not significant between formulations ($p \geq 0.05$). This result may have been influenced by the composition of the main ingredients, such as MSM, oatmeal, and cassava starch, which remain in the same color spectrum.

3.3.4. Technological cooking properties

Samples of the raw and grilled fish burgers were measured (Fig. 2). Significant effects of the variables on the cooking properties of the fish burger were evidenced from the response surface (Fig. 3). The addition of oatmeal and cassava starch favors the yield and retention of nutrients, especially in the combination between them. Verification of the cooking process is essential for foods of animal origin since micro and macronutrients change when subjected to heat treatment. Some of the water evaporates, bonds tend to break, and other compounds are desaturated, resulting in nutritional transformation and economic loss (Sheard, Nute & Chappell, 1998).

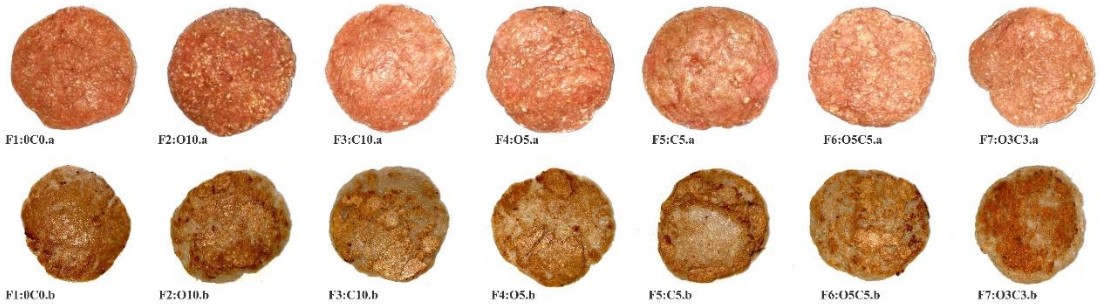

Figure 2. Fish burgers with different concentrations of oatmeal and cassava starch. Line a: raw fish burger, line b: grilled fish burger.

The cooking yield results showed a synergistic effect with better performance in samples with the addition of the two flours (F6:O5C5) ($p \leq 0.05$). A similar result regarding the inclusion of flours in fish burgers was observed in previous studies, such as the addition of chitosan in Nile tilapia fish burgers (*Oreochromis niloticus*) (Farias, Ambrósio, Vieira, Menezes & César, 2019), and in the study with the addition of oatmeal and cassava starch in the fish burger with Nile tilapia pulp (*Oreochromis niloticus*) (Braga et al., 2008). However, these results are not observed in all types of flour. In a study on the addition of wheat bran to biquara fish burgers (*Haemulon plumierii*), observed an effect of loss on yield and reduction when the addition was greater than 1% of wheat bran (Raúl et al., 2018).

The metric aspects of diameter and thickness reduction were also influenced by the addition of 10% flour (F2:O10; F3:C10). The response surface indicated inverse curves, while oatmeal affected the diameter reduction, cassava starch influenced the thickness reduction. Similar results were observed in studies with the addition of yacon flour to fish burgers of Nile tilapia (*Oreochromis niloticus*) (Zitkoski, Vendruscolo, Kuanei, Pinto & Bainy, 2019).

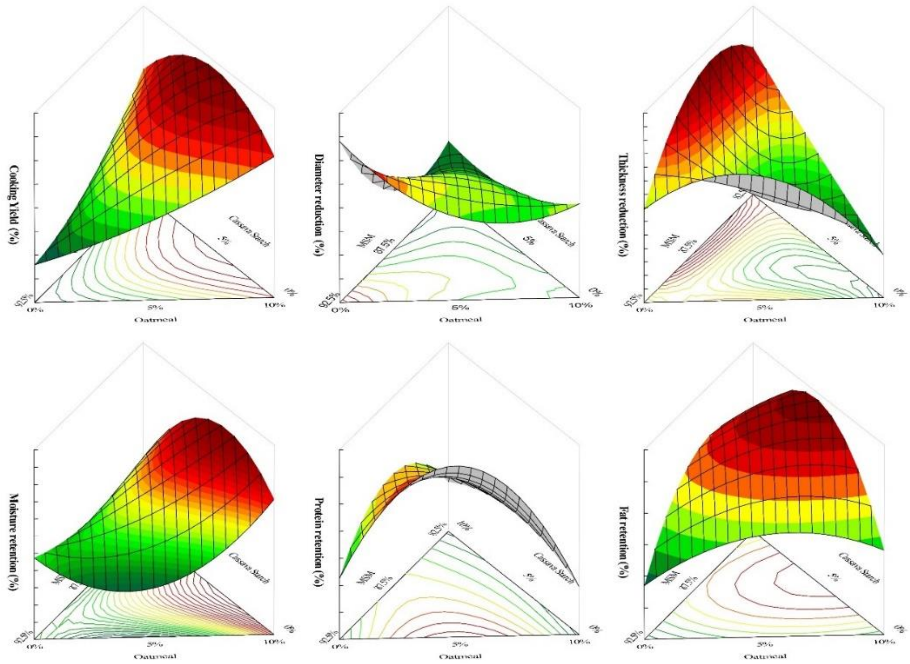

Figure 3. Response surface plots for effects of oatmeal and cassava starch on cooking properties of fish burger formulations.

There was no significant difference in moisture retention between the formulations ($p \geq 0.05$). Fat retention improved with the addition of both flours, and it performed better when combining both (F6:O5C5) ($p \leq 0.05$). Protein retention differed only in the formulation F4:O5C0 ($p \leq 0.05$) with better performance. However, when more oatmeal was added, there was a reduction in performance (F2:O10C0). The results on retention were better than those evaluated in the formulation of fish burgers with filleting waste pulp catfish (*Rhamdia quelen*) (Bochi et al., 2008). The moisture retention was similar to the study on the texture of fish burgers of Nile tilapia (*Oreochromis niloticus*), whereas fat retention values were lower (Bainy et al., 2015).

3.3.5. Optimization of multiple parameters

In this study, it was possible to predict significant effects on several factors, such as proximate composition, textural, and cooking properties. The special cubic response model allowed us to assess the influence of variables on the responses. In addition, the analysis of variance (ANOVA) confirmed the significance of the model's impact on these responses (Table 5).

Table 5. Analysis of variance of the experimental responses for MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Dependent variable response	SS	MS	R^2	p-Value
Moisture (%)	137.2482	22.8747	0.9823	0.0000*
Ash (%)	0.3349	0.0558	0.8472	0.0000*
Fat (%)	4.2788	0.7131	0.8520	0.0000*
Protein (%)	6.2939	1.0490	0.6735	0.0026*
Carbohydrate (%)	149.4580	24.9097	0.9672	0.0000*
pH	0.0095	0.0016	0.4131	0.1204^{ns}
a_W	0.0000	0.0000	0.3291	0.2567^{ns}
Cooking yield (%)	332.0694	55.3449	0.8639	0.0000*
Diameter reduction (%)	182.8260	30.4710	0.7825	0.0002*
Thickness reduction (%)	761.0008	126.8335	0.5934	0.0117*
Moisture retention (%)	108.2728	18.0455	0.4209	0.1112^{ns}
Fat retention (%)	145.5693	24.2615	0.7399	0.0005*
Protein retention (%)	208.4370	34.7395	0.7946	0.0001*
Hardness	738.8190	123.1365	0.6413	0.0050*
Cohesiveness	0.0289	0.0048	0.5552	0.0212*
Springiness	0.0487	0.0081	0.8630	0.0000*
Chewiness	245.6619	40.9437	0.6799	0.0023*
L*	102.3933	17.0655	0.2301	0.5082^{ns}
a*	16.1247	2.6875	0.1036	0.8764^{ns}
b*	106.0128	17.6688	0.1380	0.7856^{ns}

SS sum of squares, MS mean square. Statistical significance: $^*p \leq 0.05$, $^{ns}p > 0.05$.

As the objective of this study was to verify the inclusion of oatmeal and cassava starch in the fish burger formulation, the texture parameters and cooking technological properties were criteria for optimization. As the response surface for the F6:O5C5 formulation presented results close to the optimal points, the predicted values were validated with this formulation (Table 6). The optimization results will indicate the influence of the mixture on the beneficial effect on the technological properties of cooking. The lower limit and upper limit for the response parameters of the dependent variables were set at levels that ensured the integrity and intrinsic characteristics of the product.

Table 6. Predicted and observed values for optimization parameters of the fish burger formulation.

Dependent variable response	Goal	Lower limit	Upper limit	Predicted value	Experimental value (F6:O5C5)	Relative Error (%)
Cooking yield (%)	Maximize	70	100	85.41	89.38 ± 0.75	2.32
Diameter reduction (%)	Minimize	0	20	5.54	2.62 ± 0.88	41.22
Thickness reduction (%)	Minimize	0	30	18.08	23.71 ± 2.90	28.05

Moisture retention (%)	Maximize	70	100	91.13	93.53 ± 1.68	2.17
Fat retention (%)	Maximize	80	100	93.62	99.59 ± 0.29	1.18
Protein retention (%)	Maximize	80	110	99.05	99.28 ± 1.68	3.20
Hardness (N)	Is in range	10	60	34.05	24.21 ± 2.23	20.16
Chewiness (N·cm)	Minimize	5	30	13.83	9.39 ± 0.93	1.60

Results are means ± standard deviation.

Previous studies address the strong influence and improvements in the textural and yield of the fish burger when flour and starches are added (Braga et al., 2008; Coelho et al., 2007). This same effect was verified in studies with conventional products of animal origin, attenuating losses and shortening during cooking and adding juiciness (Guedes-Oliveira, Salgado, Costa-Lima., Guedes-Oliveira, & Conte-Junior, 2016; Ryszard Rezler, Krzywdzińska-Bartkowiak & Piątek, 2021). According to Talukder (2015), there are nutritional advantages to the addition of these ingredients, especially when they are used as fat substitutes, in addition to presenting an economic gain, as they are products of lesser value compared to products of animal origin. The F6:O5C5 formulation showed a 9.69% reduction in the cost of ingredients compared to the formulation without the addition of flour, at a cost of around 7.85 dollars (USD) per kg.

3.3.6. Quantitative descriptive analysis (QDA)

Sensory evaluation is a tool that allows interpreting responses to organoleptic senses and is one of the main evaluation mechanisms of food products (Dutcosky, 2011). For sensory validation, all studied formulations were analyzed under the 12 attributes raised, and the results of the quantitative descriptive analysis of the formulations are indicated in Fig. 4.

Interactions between independent variables significantly influenced the palatability of the product ($p \leq 0.05$). This result was expected, since the addition of different ingredients tends to modify the intrinsic characteristics, with effects on sensory characterization. Other studies that analyzed different concentrations of ingredients in fish burgers also showed similar results on appearance, odor, flavor, and texture (Pictures, Rocha, Ferreira & Bolini, 2015; Ali, Mansour, E-lBedawey & Osheba, 2019).

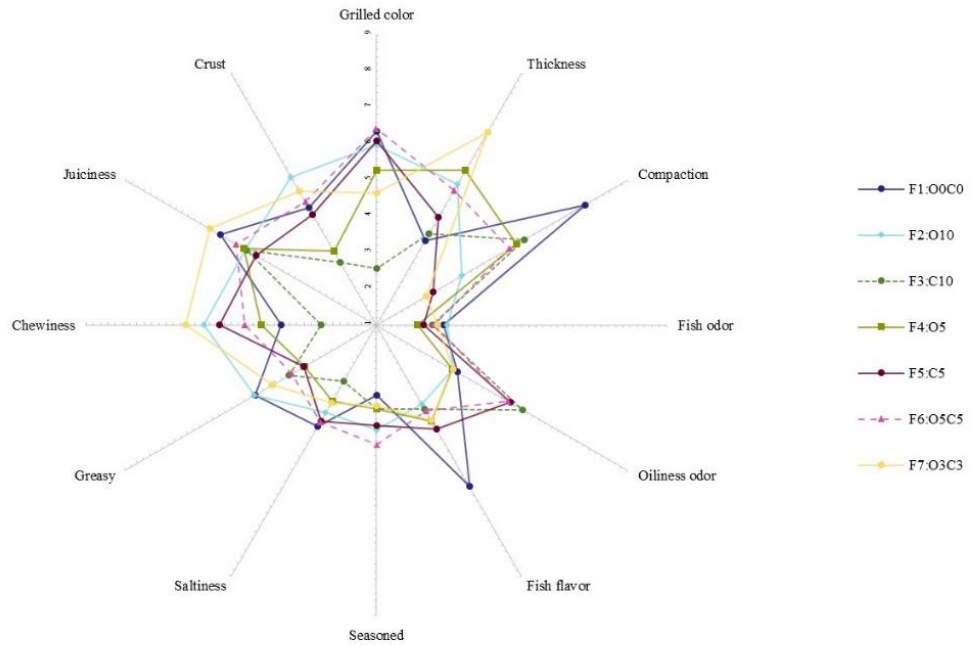

Figure 4. Descriptive Qualitative Analysis in a radar chart, for the attributes of appearance, aroma, flavor, and texture in fish burger formulations added of oatmeal and cassava starch.

The addition of flour had a significant effect on thickness, fish flavor, and compaction compared to the formulation without flour (F1:O0C0) ($p \leq 0.05$). Oatmeal had a greater effect on crust addition and thickness perception, while cassava starch had a reduction in grilled color, chewiness, and crust. However, the binary and ternary mixtures (F6: O5C5, F7: O3C3) indicated a synergistic effect on juiciness and thickness. The influence of the addition of flour was greater on appearance and texture. The study by Raúl et al. (2018), also found a greater influence on the texture and color of fish burgers of biquara (*Haemulon plumierii*) with the addition of different wheat white concentrations.

The results of descriptive parameters on fish odor, saltiness, and greasy did not show any significant difference between samples ($p \geq 0.05$).

3.3.7. Sensory affective testing

The results of the affective evaluation are presented in Table 7. The means of global acceptance did not show any significant difference ($p \geq 0.05$). The formulation F6:O5C5 presented the best score, being in the range between “liked moderately” and “liked a lot”, the other formulations showed homogeneity, in the range of “liked slightly”. Similar results were found in previous studies with convenience products based on tambaqui (*Colossoma macropomum*), Lima et al. (2020) obtained a score of 7.35 in the sensory evaluation of tambaqui fish meatballs, and according to Anjos et al. (2021), the tambaqui fish burger acceptability index was higher in the formulations enriched with green banana and chitosan biomass. The addition of oatmeal and cassava starch showed an improvement in the global acceptance of 7.44 and 7.26, respectively, in the fish burger of Nile tilapia pulp (*Oreochromis niloticus*) (Raga, Pasquetti, Bueno & Merengoni, 2008). According to Dutcosky (2011), the global acceptance test using a 9-point hedonic scale is one of the main items of sensory analysis, seeking to assess the relationship between the product/consumer and its acceptance.

Table 7. Sensory profile of the affective analysis of MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Form.	Global acceptance *(9-Point hedonic Scale)*	Purchase intention	Preference ranking*
F1:O0C0	5.67 ± 1.67^{a}	2.75 ± 0.96^{a}	5.42 ± 1.35^{b}
F2:O10	6.92 ± 1.26^{a}	3.25 ± 1.13^{a}	3.58 ± 1.92^{ab}
F3:C10	6.50 ± 1.25^{a}	2.83 ± 0.89^{a}	4.75 ± 1.75^{b}
F4:O5	6.17 ± 1.03^{a}	3.08 ± 0.63^{a}	4.25 ± 1.13^{ab}
F5:C5	6.25 ± 1.17^{a}	3.33 ± 0.83^{a}	3.92 ± 1.75^{ab}
F6:O5C5	7.58 ± 0.92^{a}	3.83 ± 0.72^{a}	3.00 ± 1.33^{a}
F7:O3C3	6.75 ± 1.50^{a}	3.67 ± 0.78^{a}	3.08 ± 1.61^{a}

Results are means ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).
*The critical difference was evaluated with the Friedman method ($p \leq 0.05$).

In the purchase intention test, the values were between “I would buy whenever I had the opportunity” and “I would buy if I had access”, the results showed no significant difference ($p \geq 0.05$). Values higher than 3.87 to 4.36 were found with the tambaqui fish burger (*Colossoma macropomum*), enriched with green banana biomass and chitosan (Anjos et al., 2021), and similar results were found in the study by Raúl et al. (2018), with a fish burger of biquara (*Haemulon plumierii*) with the addition of different wheat white concentrations, where the purchase intention was between 2.41 and 3.50.

The means of the preference ordering test showed a significant difference ($p \leq 0.05$). Formulation F6:O5C5 obtained the best position in the ranking, while the formulation without the addition of flour obtained the worst position (F1:O0C0).
The results of the sensory evaluation showed that fish burgers based on MSM of tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch were well accepted, with positive performance on the attributes of appearance, odor, flavor, texture, and hedonic tests.

3.4. Conclusion

The use of the simplex-centroid mixture design was satisfactory to estimate the effect of the pseudo-components and estimate the optimal formulation. According to the results above, the addition of oatmeal and cassava starch in the fish burger formulation showed beneficial effects on the textural, functional, and sensory properties, with desirable attributes in terms of appearance and advantages in nutritional aspects, such as the addition of fiber in the product of animal origin. Regarding optimization, the formulation with a binary combination of two flours showed results with better adjustment to the predicted optimal values, having a positive influence on the acceptance, cooking technology properties, in addition to cost reduction, as they are low-value ingredients.
The use of tambaqui MSM was satisfactory for the development of conventional products as a strategy to promote the full use of fish, and the addition of alternative ingredients can contribute to the improvement of functional and sensory properties.

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4. EFFECTS OF AVOCADO *Persea americana* Mill. BY-PRODUCT EXTRACT ON SHELF LIFE EXTENSION OF TAMBAQUI *Colossoma macropomum* FISH BURGER STORED UNDER REFRIGERATION AND FREEZING

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Presenza, L., Teixeira, B. F., Fabrício, L. F. F., Galvão, J. A., Grimaldi., R., Vieira, T. M. F. S. (2022). Effects of avocado *Persea americana* Mill. by-product extract on shelf life extension of tambaqui *Colossoma macropomum* fish burger stored under refrigeration and freezing.

Abstract

One of the main problems in the commercialization of fish products is the deterioration by enzymatic autolysis, oxidation, and microbial degradation. The objective of this study was to evaluate the bioactive capacity of extracts from avocado (*Persea americana* Mill.) by-products to extend the shelf life of fish burgers based on mechanically separated meat (MSM) of tambaqui (*Colossoma macropomum*). The evaluations of biochemical, physicochemical, and microbiological characteristics were investigated for treatment under refrigeration (4 °C) and freezing (-18 °C) storage. The addition of 2000 ppm extracts indicated beneficial effects in reducing spoilage activities, such as lipid oxidation (TBARS), formation of volatile nitrogen compounds (TVB-N), and nutritional value ($p < 0.05$). Although the synthetic antioxidant has the greatest potential to delay the formation of lipid oxidation products, the ethanolic extract of avocado seed (SEE) positively affected the reduction of spoilage activities and the lipid profile, with similar responses to the addition of sodium erythorbate (ERY) for the shelf life of fish burgers ($p < 0.05$). The analyses indicated that the addition of by-products from the agri-food industry can preserve the shelf life of fish products and be used to develop clean label products and promote more sustainable food systems.

Keywords: Antioxidant properties, Fatty acids, Freshwater, Lipid oxidation, Natural preservatives, Quality.

4.1. Introduction

Aquaculture has been considered an important food system, as the consumption of fish can bring benefits to human health, as evidenced by its relevant nutritional properties, such as the protective/reducing effect on the emergence of chronic noncommunicable diseases (NCDs) (Chen et al., 2022), and fish derived from aquaculture techniques are indicated as a viable source to feed future populations due to the potential for sustainable development when compared to extractive fishing or other methods of animal protein production (Gephart et al., 2021).

Given this context, approaches to the expansion of aquaculture production are increasingly frequent, especially when directed to native species in local consumption approaches (Short et al., 2021). For example, tambaqui (*Colossoma macropomum*), a native species of the Amazon River basin originating in the South America region, is a fish widely consumed mainly in the Amazon region; however, the consumption of the species has become popular and is currently the most produced native species in Brazil (Hilsdorf et al., 2021; PeixeBR, 2022).

However, the sustainability of the aquaculture sector is also related to the quality management and conservation of fish products, since fish are conventionally stored and marketed in ice or freezing, and the cold chain is not always strictly maintained (Mercier et al., 2017; Wu & Hsiao, 2021); therefore, methods aimed at prolonging shelf life, preserving freshness, and ensuring hygienic-sanitary safety of fish products are essential, as they are foods highly susceptible to the formation of nitrogenous compounds due to the number of amino acids, lipid oxidation due to the presence of unsaturated fatty acids with multiple double bonds and microbial development favored by the high rate of water activity (Sheng & Wang, 2020; Nie et al., 2022).

Approaches to preservation techniques based on natural products such as essential oils (EOs), active packaging, and edible films are increasingly present, mainly because consumers seek to reduce the consumption of synthetic compounds (Yu et al., 2020; Baptista, Horita & Sant'Ana, 2020; Xu et al., 2021). Most plants are rich in phenolic compounds that have active properties through the neutralization of free radicals, potentially reducing spoilage biochemical reactions and antimicrobial activity (Maqsood, Benjakul & Shahidi, 2012; Shen et al., 2022).

Avocado (*Persea americana* Mill.) is an important agri-food industry product consumed all over the world, usually sold *in natura*, but it can also be presented in the form of juice and oil, which generates a considerable amount of waste, mainly the peel and the seed, that can represent up to 30% of the fruit weight (Melgar et al., 2018). Previous studies have pointed out the feasibility of using avocado residues as a source of bioactive compounds. The peel and seed are rich in polyphenols and have a potential antioxidant effect, which can provide substantial improvements in food storage (Salazar-López et al., 2020; Figurosa et al., 2021).

In the present study, aqueous-ethanolic extracts of avocado (*Persea americana* Mill.) were incorporated into formulations of fish burgers based on mechanically separated meat (MSM) of tambaqui (*Colossoma macropomum*) submitted to different storage methods for up to 90 days. The potential preservative effect of avocado by-products was monitored by physicochemical, analytical, and microbiological analyses compared to a control lot and synthetic antioxidant.

4.2. Material and methods

4.2.1. Chemicals

All chemicals used were of analytical grade. Ethanol (PubChem CID: 702) (Êxodo®. Sumaré, Brazil); petroleum ether (Êxodo®, Brazil); trichloroacetic acid (TCA) (Merck®, Germany) (PubChem CID: 6421); titriplex (EDTA) (Merck®, Germany) (PubChem CID: 6049); propyl 3,4,5-trihydroxybenzoate (PG) (PubChem CID: 4947); 1,1,3,3-tetraethoxypropane (TEP) (Sigma-Aldrich®, Germany) (PubChem CID: 67147); 2-thiobarbituric acid (TBA) (Sigma-Aldrich®, Germany) (PubChem CID: 2723628); Folin-Ciocalteau reagent 2 M (Êxodo®, Brazil); gallic acid (PubChem CID: 370) (Sigma-Aldrich®, Germany); sodium carbonate (PubChem CID: 10340) (Sigma-Aldrich®, Brazil); boric acid (PubChem CID: 7628) (LS Chemicals®, Brazil); sulfuric acid (PubChem CID: 1118) (LS Chemicals®, Brazil); magnesium oxide (PubChem CID: 14792) (LS Chemicals®, Brazil); sodium erythorbate (PubChem CID: 23683938) (QuimisulSC®, Brazil).

Microbiological medium: Petrifilm EC (3 M, USA); Petrifilm Environmental Listeria (3 M, USA); Trypticase Soy Agar (TSA) (KASVI®, Spain); Brilliant Green (Scharlau®, Spain); Xylose Lysine Deoxycholate Agar (XLD) (KASVI®. Spain); Baird Parker (Scharlau®, Spain) containing egg yolk (Dinâmica®, Brazil); Brain Heart Infusion (BHI) (Acumedia®, USA).

4.2.2. Raw materials and MSM preparation

Avocado (*Persea americana* Mill.) peels and seeds were kindly provided in June 2020 by *Novo Mundo* farm (São Tomás de Aquino/MG, Brazil). After harvesting and processing, the peels and seeds were washed in water and submitted to sun drying for 4 hours.

Fifteen samples (N = 15) of tambaqui (*Colossoma macropomum*) were supplied by a local producer (Piracicaba/SP, Brazil). The samples, from the same batch and produced in an aquaculture system, were received frozen by the Fish Processing Laboratory ESALQ/USP (Piracicaba/SP, Brazil) and had an average weight of 1.2 kg. Then, the strips were thawed, and the head and skin were removed before obtaining mechanically separated meat (MSM) in a mincer (Model 250C, High Tech®, Brazil). The MSM was homogenized and stored in packages containing 1 kg and frozen at -18 °C. Other ingredients, including oatmeal, cassava starch, NaCl, monosodium glutamate and spices, were also purchased from the local market (Piracicaba/SP, Brazil).

4.2.3. Preparation of extracts

Obtaining the avocado (*Persea americana* Mill.) by-product extracts followed an optimized method in an additional study (unpublished data), two extracts were obtained: avocado peel extract and avocado seed extract. The peels and seeds were lyophilized (Freeze-drying L101, Liotop®, Brazil) for approximately 36 hours. After drying, the samples were crushed and sieved (2 mm), and then 2.25 g of avocado peel powder in 10 ml of ethanol (40%) and 5 g of avocado seed powder in 10 ml of ethanol (20%) were homogenized and ultrasonicated (Ultrasonic bath LSUC2, Logen®, Brazil) at 135 W for 15 minutes at 30 °C. Subsequently, the samples were centrifuged (Universal 320 R, Hettich®, UK) at 5000 RCF for 15 minutes. The extract was obtained by vacuum filtration on qualitative filter paper (Whatman®, Germany). The extract obtained was quickly collected and applied in the preparation of fish burgers.

4.2.3.1. Determination of total phenolics

The determination of the total phenolic content of the extracts was performed using the Folin-Ciocalteau method and expressed in gallic acid equivalents (mg GAE/g) (Woisky & Salatino, 1998; Singleton et al., 1999). An amount of 2 M Folin-Ciocalteau (1:10 v/v) was added to 0.5 ml of extract. After 5 min, 2 ml of 4% Na_2CO_3 was added to the mixture, which was incubated for 10 min at room temperature.

4.2.4. Fish Burger preparation and sampling

The fish burgers were prepared according to the optimized formulation in a previous study (Presenza et al., 2022) using MSM (82.5%), oatmeal (5%), cassava starch (5%), NaCl (1.5%), monosodium glutamate (0.2%), white pepper (0.1%), garlic powder (0.3%), onion powder (0.4%) and ice (5%).

The ingredients were homogenized in an industrial cutter and subsequently subjected to 4 treatments with the addition of 2000 ppm of sodium erythorbate (ERY), 2000 ppm of peel extract (PEE), 2000 ppm of seed extract (SEE), and control (CON). The fish burgers were portioned into 90 g pieces, molded to 10 cm in diameter and packed in low-density polyethylene bags. Then, they were stored for 14 days in an expository freezer with white light at 4 °C and 90 days at -18 °C in a vertical freezer. The biochemical and physicochemical properties were evaluated on days 0, 3, 7, 14, 30, 60 and 90, and the proximate composition, microbiological parameters, and lipid profile were analyzed on the initial and final days for both storage treatments, as summarized in Figure 1.

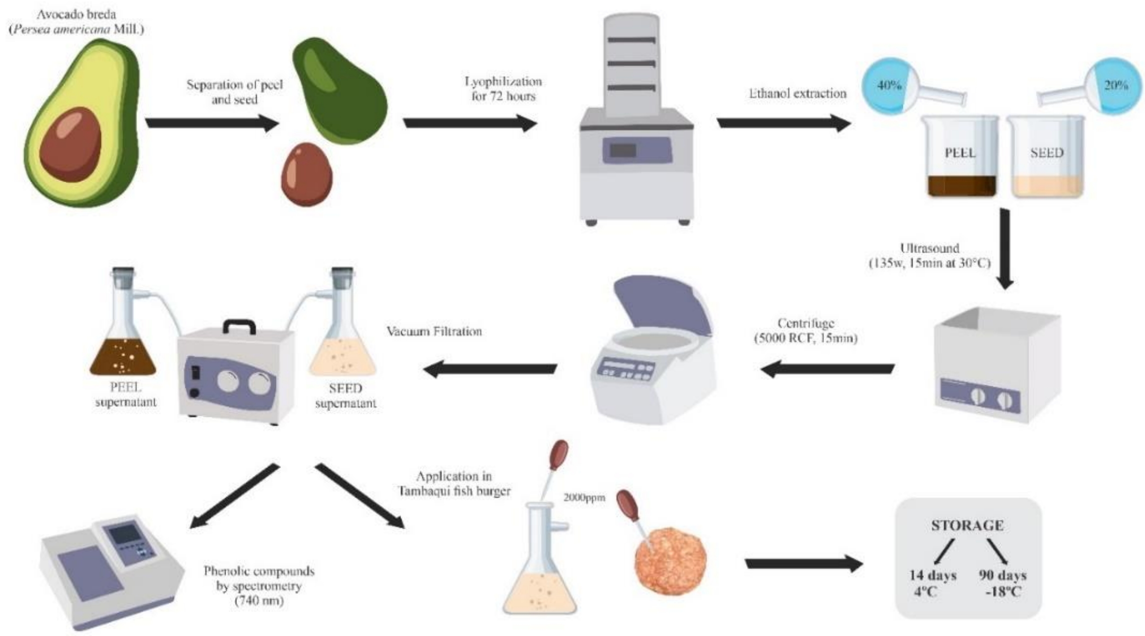

Figure 1. Experimental design, a scheme for obtaining ethanolic extract, from avocado (*Persea americana* Mill.) by-product and study of application and storage sampling in tambaqui (*Colossoma macropomum*) fish burgers.

4.2.4.1. Microbiological analyses

To determine microbiological safety, 25 g was taken for analysis of each formulation in triplicate (N = 3). Samples were homogenized (Stomacher ITR MC-1204, Brazil) with 225 mL of 0.1% peptone salt solution for enumeration of total coliforms, *Escherichia coli,* and coagulase-positive staphylococci. Another 25 g sample was homogenized in 225 mL of 1% buffered peptone solution (pre-enrichment) for analysis of *Salmonella* spp.

The analysis of total coliforms and *Escherichia coli* was carried out following AOAC® Method 998.08:2002 (3 M Petrifilm), environmental *Listeria sp.* According to the AOAC® Performance Test Method (3 M Petrifilm - Certificate Number 030601), coagulase-positive staphylococci were identified according to ISO 6888-1:2021. Both results are expressed in CFU/g^{-1}, and *Salmonella* spp. according to ISO 6579:2017 expressed as absence or presence per 25 g.

4.2.5. Physicochemical analyses

4.2.5.1. Proximate composition

For analysis of the proximate composition of fish burger formulations, the tests were performed in triplicate (N = 3) on raw samples by AOAC official methods (2005). Crude protein was determined by the Kjeldahl method (using N x 6.25 as a conversion factor) (TE-036, Tecnal®, Brazil); the fat was extracted with hexane in a Soxhlet extractor (TE-188, Tecnal®, Brazil); moisture was determined by the gravimetric method in an oven with air circulation at 105 °C (TE-394, Tecnal®, Brazil); the ash content was determined by incineration in a muffle furnace at 550 °C (Quimis®, Brazil). The carbohydrate level of the fish burgers was determined by subtracting the moisture, protein, fat, and ash percentages from 100. The energy value was determined based on 100 g by multiplying the crude protein and carbohydrates by 4 and fat by 9.

4.2.5.2. pH measurement

The pH was measured on a raw fish burger using a pH meter (Tec-3MP, Tecnal®, Brazil). Measurements were performed in triplicate (N = 3).

4.2.5.3. Total volatile basic nitrogen (TVB-N)

The determination of TVB-N was performed in triplicate (N = 3) following the methods described by Silva et al. (2008), with some modifications. Fifty grams of fish burger samples were homogenized (IKA T18 basic, Ultra-Turrax®, Germany) with 150 ml of trichloroacetic acid (TCA) (50 g/L), and the vacuum filtered supernatant was distilled with a Kjeldahl micro distillation unit (TE-036, Tecnal®, Brazil) with 1 g of magnesium oxide. Then, 20 ml of boric acid (40 g/L) containing 4-5 drops of the mixed indicator was added. Then, the solution was evaluated with 0.1 N sulfuric acid. The TVB-N values were expressed in units of mg nitrogen (mg N/100 g^{-1}), according to the following equation (1):

$$\text{TVB-N (mg N/100g}^{-1}) = \frac{14 \text{ x } (150+\text{A}) \text{ x V x F x N x } 100}{\text{Va x P}} \tag{1}$$

Where, N = sulfuric acid normality; V = volume of sulfuric acid in the titration; F = sulfuric acid correction factor; Va = filtered aliquot volume; P = sample mass, and A = sample moisture.

4.2.5.4. Measurement of lipid oxidation

Thiobarbituric acid reactive substances (TBARS) were measured in triplicate (N = 3) following Vyncke (1970) with some modifications. For the determination, 5 g of fish burger samples were homogenized (IKA T18 basic, Ultra-Turrax®, Germany) with 15 ml of trichloroacetic acid (TCA) solution (7.5%). The supernatant was filtered through qualitative filter paper (Whatman®, Germany), and then 5 ml of 2-thiobarbituric acid (TBA) was added to the filtrate and placed in a steam bath (NT 269, Nova Técnica®, Brazil) for 40 min. After bubble removal, the absorbance (A_s) was measured at 538 nm against the dh_2o blank, and the reagent blank was run on absorbance (A_b) at 600 nm (UV mini-1240, Shimadzu®, Japan). TBARS values were expressed in mg of malonaldehyde (MDA/kg) of the sample obtained by the formula.

$$\text{TBARS} = \frac{50 \, x \, (A_s - A_b)}{200} \tag{2}$$

4.2.5.5. Gas chromatography-mass spectrometry analysis

The fatty acids present in the raw fish burgers were analyzed from 3 samples (N = 3) separated by a Series Gas Chromatography System (Agilent Technologies, Santa Clara, CA) with a DB-23 capillary column (50% cyanopropyl-methylpolysiloxane) with dimensions of 60 m, 0.25 mm, and 0.25 μm film. The chromatograph operating conditions were as follows: column flow 1.00 mL/min, linear velocity 24 cm/s, detector temperature 280 °C, injector temperature 250 °C, oven temperature, 110 °C for 5 min, 110 to 215 °C (5 °C/min), 215 °C for 24 min, carrier gas (helium), and the volume injected was 1.0 μL. Fatty acids were quantified by comparing retention times to peaks on a standard curve. The quantitative determination of fatty acids was performed by area normalization and expressed in percentage by mass according to the method described by the AOCS (2009).

4.2.5.6. Instrumental color measurement

Color measurement of fish burgers was determined using a colorimeter (Chroma Meter CR-400, Konica Minolta®, Japan), measuring an area of 8 mm in diameter. The *L** (luminance ranging from black to white), *a** (green to red), and *b** (blue to yellow) color coordinates were measured. The analysis was performed in triplicate (N = 3) with three readings on each side, totaling 18 readings per treatment.

4.2.6. Statistical analysis

The values of the different parameters were expressed as the mean and standard deviation using analysis of variance (ANOVA), and Tukey's test was applied to compare the means with a significance level of 5%. Data were analyzed using Statistica® 13.3 software from TIBCO (Palo Alto, California, USA).

4.3. Results and Discussion

4.3.1. Total phenolic content (TPC) in avocado by-products

Avocado (*Persea americana* Mill.) is a tropical fruit widely spread in the diet of several populations and has been showing high development in propagation to meet a growing level of consumers; in this context, the residues of avocado production are evaluated when the most appropriate destination is in terms of the economic and sustainable dimension (Del Castillo-Llamosa et al., 2021). Previous studies have evaluated potential bioactive effects on avocado peel and seed, in an evaluation of total phenolics in avocado peel at a concentration of 1:20 (sample/solvent),

conditions: 70 °C, 15 min, EtOH 75% a value of 29.7 (mg GAE/g peel) was observed, and in a seed evaluation at a concentration of 1:15 (sample/solvent) conditions: 40 °C, 20 h, EtOH 80%, a value of 63.19 (mg GAE/g seed) (Velderrain-Rodríguez et al., 2021; Figueroa et al., 2021).
These results were slightly higher than those observed in this study (Table 1); however, it is necessary to observe that the concentrations and extraction time were higher, and the results of the total phenolic composition were also higher for the seed (23.5 mg GAE/mL) than for the peel (12.1 mg GAE/mL) ($p < 0.05$).

Table 1. Total phenolic compounds of the avocado (*Persea americana* Mill.) extract by-products.

Samples	Temperature (°C)	Time (min)	Solvent (% EtOH)	Sample-solvent ratio (g/10 mL)	TPC (mg GAE/mL)
PEEL	30	15	40	2.25	12.1 ± 0.18^{b}
SEED	30	15	20	5	23.5 ± 0.16^{a}

TPC: Total phenolic content is expressed as mg of gallic acid equivalent (GAE). Values are expressed as the means ± standard deviation. Different letters next to the means indicate a significant difference ($p < 0.05$).

An additional study (unpublished data) was performed to evaluate the same batch of avocados (*Persea americana* Mill.) and found similar responses for phenolic content, indicating that the main secondary constituents of the peel were 3,4-hydroxybenzoic acid, syringic acid, and caffeic acid, while for the seed, they were 3,4-hydroxybenzoic acid, catechin, and syringic acid. These results corroborate the potential application of avocado extracts as preservative agents in food products, mainly to reduce lipid oxidation.

4.3.2. Antimicrobial activity of extracts

Microbiological analyses were performed on three fish burger samples for each treatment during each storage treatment's initial and final period (N = 3). The groups of bacteria analyzed were defined based on the potential biological risk based on Brazilian (Brazil, 2019) and European (EC, 2005) resolutions, which establish standards on the microbiological safety of fish-based products. The results indicated that all treatments remained within the established limits after the end of each storage treatment (Table 2).

Table 2. Microbiological evaluation of raw fish burgers based on MSM of tambaqui (*Colossoma macropomum*) with different active compounds under different storage treatments.

Storage days/ Treatments	Initial day	14 (4 °C)	90 (-18 °C)
Coagulase-positive staphylococci (CFU/g)			
CON	≤ 1.0×10^1	≤ 1.0×10^1	≤ 1.0×10^1
ERY	≤ 1.0×10^1	≤ 1.0×10^1	≤ 1.0×10^1
PEE	≤ 1.0×10^1	≤ 1.0×10^1	≤ 1.0×10^1
SEE	≤ 1.0×10^1	≤ 1.0×10^1	≤ 1.0×10^1
Salmonella spp. (in 25 g)			
CON	absent	absent	absent
ERY	absent	absent	absent
PEE	absent	absent	absent
SEE	absent	absent	absent
Escherichia coli (CFU/g)			
CON	absent	absent	absent
ERY	absent	absent	absent
PEE	absent	absent	absent
SEE	absent	absent	absent
Total coliform (CFU/g)			
CON	6.0×10^1	1.0×10^3	1.0×10^1
ERY	3.7×10^2	1.0×10^3	1.0×10^1
PEE	1.0×10^3	2.0×10^3	2.0×10^1
SEE	4.6×10^2	1.0×10^3	≤ 1.0×10^1
Listeria spp. (CFU/g)			
CON	6.0×10^1	≤ 1.0×10^1	≤ 1.0×10^1
ERY	1.3×10^3	1.0×10^1	≤ 1.0×10^1
PEE	5.1×10^2	1.0×10^1	≤ 1.0×10^1
SEE	5.3×10^2	≤ 1.0×10^1	≤ 1.0×10^1

CFU/g - Colony forming unit per gram of product. (CON: control, ERY: erythorbate treatment, PEE: peel extract treatment, SEE: seed extract treatment).

Regarding coagulase-positive staphylococci, all tests were negative, both between treatments and during the storage treatments. This result may be related to the hygienic-sanitary quality of the raw material, which did not reveal contamination since the initial sampling. In addition, typical colonies of *Salmonella spp.* were also not identified between treatments and storage.

In the analysis of enterobacteria, typical colonies of *Escherichia coli* were not identified, both between treatments and during the storage period; however, the number of typical colonies of coliforms was lower for the control treatment (CON) on the initial day of storage treatments. This result may indicate contamination of

antioxidants added to the formulation, both from synthetic and ethanolic avocado extracts. After 14 days of storage at 4 °C, there was an increase in colonies of typical coliforms for all treatments, indicating that there was no antimicrobial effect for enterobacteria. In the freezing storage treatment at -18 °C, the samples showed a reduction in the number of typical coliform colonies, a result already expected, since the temperature has a significant effect on microbial growth (Huang et al., 2011); therefore, comparing different temperatures, it was possible to identify a greater effect of temperature on microbial control than the extracts used.
Similar results were observed for the analysis of *Listeria spp*., which presented a greater number of typical colonies in the initial sampling; however, for this group, a reduction in the number of colonies was observed after 14 days under refrigeration, and after 30 days of freezing, no typical colonies were identified.
Previous studies investigating the potential antimicrobial effect of ethanolic plant extracts in fish products also observed an increase in CFU counts at 4 °C storage (Mayeli et al. 2019; Miranda et al., 2021); however, the values were not superior to those of the control. Regarding the microbiological quality of fish burgers stored at -18 °C, a study using guabiroba peel (*Campomanesia xanthocarpa*) in tilapia burgers (*Oreochromis niloticus*) also showed a low number of colonies for coagulase-positive staphylococci, *Salmonella spp*. and *Escherichia coli* (Cristofel et al., 2021). Although other studies with natural extracts indicate a high proportion of phenolic compounds in microbial cell permeability as a control agent (Seifzadeh & Khorasgani, 2020; Lekshmi et al., 2021), in this study, there were no effects on reducing microorganisms compared to the formulation of the control treatment.

Table 3. Color parameters (*L**, *a**, and *b**) of raw fish burgers based on MSM of tambaqui (*Colossoma macropomum*) with different active compounds under different storage treatments.

Storage days/Samples	Initial day	3 (4 °C)	7 (4 °C)	14 (4 °C)	30 (-18 °C)	60 (-18 °C)	90 (-18 °C)
*L**							
CON	69.74 ± 1.20^{A}	68.53 ± 1.86^{A}	68.00 ± 0.65^{A}	66.39 ± 0.97^{AB}	69.40 ± 0.90^{A}	69.04 ± 0.60^{A}	64.69 ± 1.14^{aB}
ERY	69.27 ± 1.95^{A}	68.00 ± 0.65^{A}	66.85 ± 0.53^{A}	66.18 ± 0.84^{A}	68.15 ± 1.81^{A}	67.04 ± 1.67^{A}	60.75 ± 0.58^{bB}
PEE	69.26 ± 1.76^{A}	68.30 ± 1.39^{A}	66.40 ± 0.49^{AB}	66.41 ± 0.34^{AB}	68.74 ± 1.36^{A}	68.22 ± 0.96^{A}	63.09 ± 0.49^{abB}
SEE	68.60 ± 1.14^{A}	68.50 ± 1.22^{A}	66.78 ± 1.42^{AB}	66.77 ± 0.27^{AB}	68.60 ± 1.10^{A}	68.61 ± 1.07^{A}	63.92 ± 0.84^{aB}
*a**							
CON	8.01 ± 0.66^{A}	2.99 ± 0.37^{cD}	5.44 ± 0.40^{abBC}	7.42 ± 0.35^{A}	6.22 ± 0.39^{B}	5.44 ± 0.11^{BC}	4.36 ± 0.60^{CD}
ERY	8.83 ± 0.49^{A}	4.64 ± 0.40^{bC}	5.32 ± 0.43^{bC}	6.02 ± 0.68^{BC}	6.98 ± 0.43^{B}	6.12 ± 0.36^{BC}	4.91 ± 0.04^{C}
PEE	8.14 ± 0.50^{A}	4.68 ± 0.68^{bC}	6.20 ± 0.33^{aBC}	7.24 ± 0.44^{A}	6.56 ± 0.49^{A}	5.99 ± 0.48^{BC}	4.40 ± 0.47^{C}
SEE	8.29 ± 0.29^{A}	6.56 ± 0.49^{aB}	6.50 ± 0.33^{aB}	6.70 ± 0.45^{B}	6.59 ± 0.36^{B}	5.88 ± 0.43^{BC}	4.89 ± 0.30^{C}
*b**							
CON	13.32 ± 0.63	14.62 ± 0.89	14.80 ± 0.45	15.04 ± 0.53	13.44 ± 0.41	13.57 ± 0.20	14.76 ± 0.76
ERY	13.54 ± 0.36	13.80 ± 0.24	14.43 ± 0.71	14.16 ± 0.74	13.38 ± 0.52	13.22 ± 0.67	13.35 ± 0.78
PEE	13.70 ± 0.82	14.15 ± 0.86	14.12 ± 0.71	14.71 ± 0.65	13.65 ± 0.95	13.60 ± 1.07	14.56 ± 0.72
SEE	13.85 ± 0.61	13.67 ± 0.65	13.95 ± 0.53	14.33 ± 0.73	13.75 ± 0.61	13.66 ± 0.61	14.48 ± 0.82

Values are expressed as the means ± standard deviation. Different lowercase letters in each column indicate a significant difference between treatments ($p < 0.05$), and different uppercase letters in each row indicate a significant difference between storage conditions ($p < 0.05$). (CON: control, ERY: erythorbate treatment, PEE: peel extract treatment, SEE: seed extract treatment).

4.3.3. Effects of extracts on instrumental measures of fish burger color

Color is an important parameter to assess the quality and consumer acceptance of a product since deterioration affects the physicochemical properties with consequent color change that can lead to product rejection (Clydesdale, 1991; Andrés-Bello et al., 2013). The fish burgers were evaluated for color change (L^*, a^*, and b^*) during the refrigeration and freezing storage treatments, where L^* and a^* values differed significantly between treatments and storage period ($p < 0.05$), while the value of b^* showed no significant difference (Table 3).

The L^* values that respond to the luminosity of the samples were significantly different between treatments (CON, ERY, PEE and SEE) in the final stage of freezing storage (-18 °C) after 90 days and showed a decrease in luminosity compared to the responses observed for the other storage periods ($p < 0.05$).

For the a^* coordinate, the values were significantly different between the refrigeration treatments (4 °C). The responses indicated a critical phase of color change on day 3 (4 °C), where the decrease in intensity was abrupt for this coordinate. The treatments CON, ERY, and PEE and the inclusion of seed extract (SEE) showed better results in color maintenance ($p < 0.05$), and on day 7 (4 °C), the values increased again and were better preserved in treatments with ethanolic extracts of avocado (*Persea americana* Mill.) (PEE and SEE) ($p < 0.05$). A reduction in the intensity of the a^* coordinate was also observed for all treatments during the storage periods ($p < 0.05$).

The results indicated that ethanolic extracts from avocado by-products (PEE and SEE) were significantly more effective in maintaining the initial color of fish burgers during refrigeration (4 °C) and freezing (-18 °C) storage ($p < 0.05$). A previous study with Nile tilapia burgers (*Oreochromis niloticus*) also identified better color maintenance results in the use of ginger (*Zingiber officinale* Roscoe) essential oil (EOs) than in the treatment with sodium erythorbate (Mttje et al., 2019). Similar results were found in the study by Rico et al. (2020), where the addition of aqueous extracts of fennel (*Crithmum maritimun*) also favored the maintenance of the color of Atlantic horse mackerel (*Trachurus trachurus*) burgers during refrigeration storage (4 °C).

4.3.4. Biochemical changes and inhibition of oxidation products

The values of secondary compounds of lipid oxidation through 2-thiobarbituric acid reactive substances (TBARS) were significantly different between treatments and increased during refrigeration (4 °C) and freezing (-18 °C) storage ($p < 0.05$), although values were lower for frozen samples, which was expected since temperature slows down biochemical processes, responses to effects were similar between different storages (Figure 2). The treatment with sodium erythorbate (ERY) showed better results in both storage treatments, with initial values of 0.25, 0.37, and 0.44 mg (MDA/kg^{-1}) for the end of the refrigeration and freezing treatments, respectively. The addition of ethanolic extracts of avocado (*Persea americana* Mill.) by-products showed significantly different responses, and the seed extract (SEE) obtained lower TBARS values ($p < 0.05$), with an initial value of 0.26, and 0.72 and 0.65 mg (MDA/kg^{-1}), for the treatment's end in refrigeration and freezing, respectively, and the treatment with peel extract (PEE) of 0.27, and 0.85 and 0.75 mg (MDA/kg^{-1}), respectively.

At the end of both storage treatments, the application of synthetic and natural antioxidants showed significantly lower values than the control (CON) ($p < 0.05$), where the TBARS value was initially 0.29 and 0.99 and 0.85 mg (MDA/kg^{-1}) for the end of the refrigeration and freezing treatments, respectively. Only the control treatment (CON) at 14 days under refrigeration (4 °C) showed a critical TBARS value above 0.9 mg (MDA/kg^{-1}), indicating a high degree of lipid oxidation products.

Biochemical processes that follow the *post-mortem* stage result in the attenuation of the quality of the fish products, being one of the main factors of losses in commercialization, which generates great concern for the fish products industries. Among these processes, the formation of free radicals by lipid auto-oxidation, in response to the interaction of fatty acids with atmospheric oxygen, generates a considerable loss of sensory quality, leading to product rejection, especially in ictic species with high unsaturated fatty acid contents (Nie et al., 2022). Although the results of the addition of the synthetic antioxidant were more effective, the findings on the responses of avocado extracts prove the ability of natural extracts to delay the lipid oxidation of fish-based products with effects on the maintenance of sensory properties. A study by Gai et al. (2014) investigated the application of an ethanolic extract of red grape pomace (*Vitis vinifera* L.) on rainbow trout (*Oncorhynchus* *mykiss*) at a similar concentration (1% and 3%) and showed a significantly lower TBARS value than the control after 6 days of storage (4 °C). These results corroborate the fact that agri-food industry residues can be a tool in the development of clean label products with more sustainable processes, and further investigations can validate more competitive responses to replace synthetic compounds.

Another indicator of autolytic deterioration and the quality of fish products is the total volatile basic nitrogen (TVB-N) content, which comprises two main compounds, methylamines and trimethylamine (produced via autolytic enzymes and spoilage bacteria), and is characterized by a strong odor that leads to rejection of fish products (Moosavi-Nasab et al., 2021). Changes in TVB-N values between treatments and different storage periods are shown in Figure 2. The initial levels of TVB-N of tambaqui (*Colossoma macropomum*) fish burgers were 20.52, 17.66, 17.28, and 17.00 mg (N/100 g^{-1}) between treatments CON, ERY, PEE, and SEE, respectively. These values are already considered high for freshness and significantly increased after the completion of both storage treatments ($p < 0.05$). For refrigeration storage, the final values after 14 days at 4 °C were 36.17, 22.83, 32.89, and 22.94 mg (N/100 g^{-1}), between treatments CON, ERY, PEE, and SEE respectively, and for the treatment at -18 °C after 90 days, 34.36, 29.63, 32.17 and 29.38 mg (N/100 g^{-1}), between treatments CON, ERY, PEE and SEE, respectively.

Quality standards for fish-based products, in general, are not well described; however, Brazilian (Brazil, 2017) and European (EC, 2005) legislation establish an acceptability limit for TVB-N in fresh or frozen fish of 30 mg (N/100g^{-1}) for most fish species. Based on this critical value, the acceptable shelf life for the analyzed samples of tambaqui (*Colossoma macropomum*) fish burgers was 14 days (4 °C) and 90 days (-18 °C) for the treatments with sodium erythorbate (ERY) and seed extract (SEE), while the control (CON) and peel extract (PEE) samples showed TVB-N values above the acceptability limit after 14 days (4 °C) and 90 days (-18 °C).

The increase in TVB-N content after the initial day was significant for the treatment under refrigeration and freezing; however, storage at 4 °C indicated greater development of nitrogenous bases, which was evidenced in the pH analysis, which showed a more significant difference between treatments ($p < 0.05$). This is mainly due to the formation of free amines due to the protease of autolytic enzymes and endogenous spoilage bacteria that led to the formation of alkaline compounds that became more evident after 14 days at 4 °C, where the control treatment (CON) and peel extract (PEE) obtained the highest pH values of 6.38 and 6.64, respectively, while the values for treatment with sodium erythorbate (ERY) and seed extract (SEE) were 5.83 and 5.84. These results indicate a significant effect of the addition of avocado seed extract (SEE) in controlling the formation of volatile bases ($p < 0.05$), with similar results to the treatment with sodium erythorbate (ERY) ($p > 0.05$).

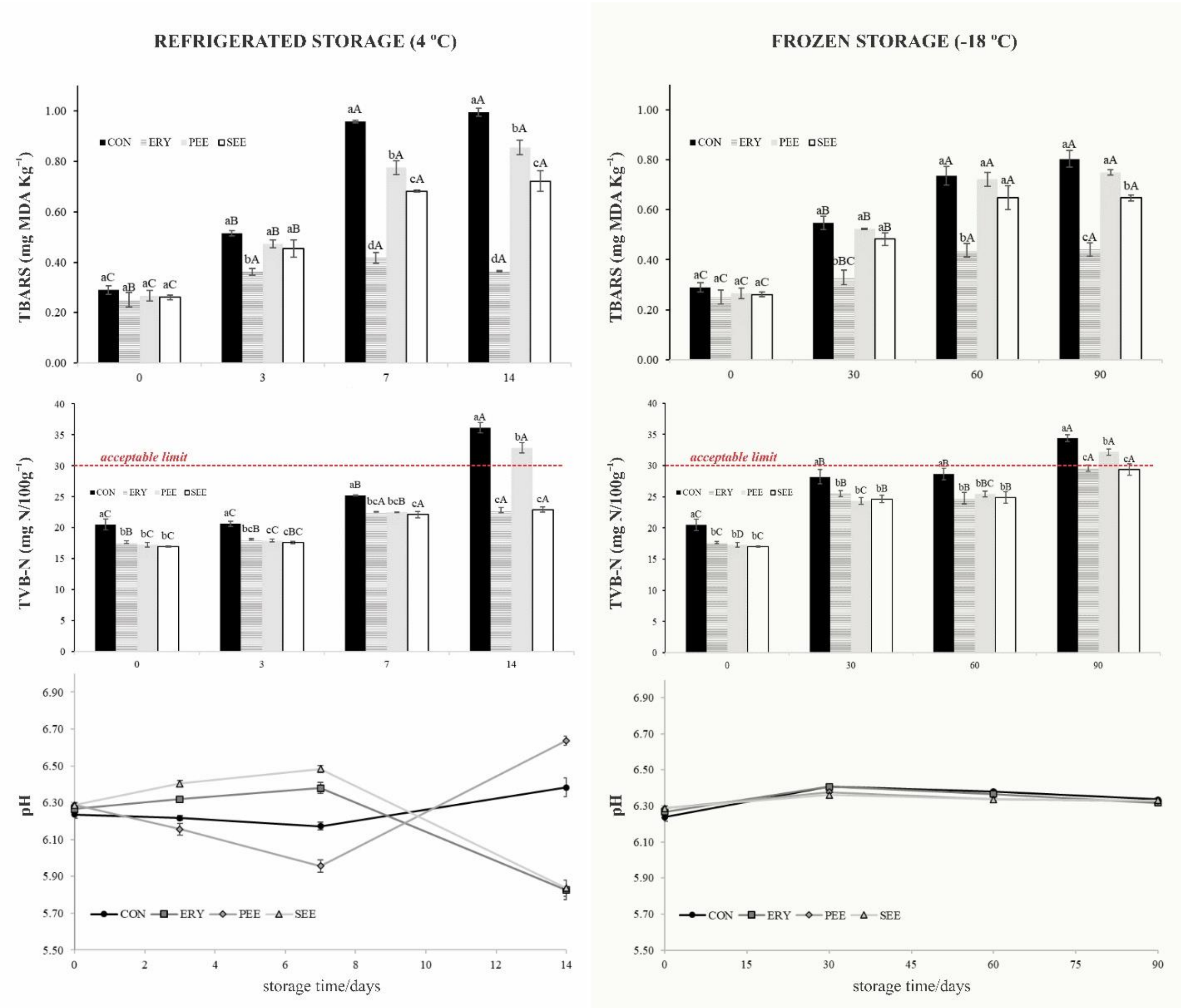

Figure 2. Lipid oxidation TBARS, total volatile nitrogenous base TVB-N, and pH of raw fish burgers based on MSM of tambaqui (*Colossoma macropomum*) with different active compounds under different storage treatments. Values are expressed as the means with a standard deviation bar. Different lowercase letters indicate a significant difference between treatments ($p < 0.05$), and different capital letters indicate a significant difference between storage days ($p < 0.05$). (CON: control, ERY: erythorbate treatment, PEE: peel extract treatment, SEE: seed extract treatment).

Similar effects were found for the treatment for frozen samples (-18 °C), and the increase in TVB-N was more pronounced after 30 days of storage, followed by an increase in pH for both treatments; however, the temperature effect began to develop more slowly with pH stability responses after 90 days. The effects of improving biochemical properties, reducing lipid oxidation, and extending the shelf life of fish products with natural plant extracts have also been reported in other studies (Gai et al., 2015; Baptista et al., 2020; Lekshmi et al., 2021).

The addition of different active compounds as preservatives indicated significant effects for both storage treatments (Table 4), and previous studies that evaluated the shelf life of tambaqui (*Colossoma macropomum*) products observed similar responses. In the meatball study, the pH was higher in the addition of oregano (*Origanum vulgare*) OEs (6.40) than in the control (6.05), and investigation of pH and in fillets stored for 22 days on ice obtained a TVB-N value of 23 mg (N/100g^{-1}) and pH 6.7 after storage, and fillets submitted to *sous vide* treatment showed a value to TBARS 0.60 mg (MDA/kg^{-1}) after 60 days at 1 °C (Kato et al., 2017; Silva et al., 2018; Lima et al., 2020).

Table 4. Analysis of variance of biochemical and physicochemical responses for the application of different active compounds in the formulation of fish burgers based on MSM obtained from tambaqui (*Colossoma macropomum*).

Storage/	Refrigeration (4 °C)					Freezing (-18 °C)				
Treatments	SS	MS	R^2	F test	p Value	SS	MS	R^2	F test	p Value
TBARS										
CON	1.07	0.36	1.00	1348.60	0.00*	0.48	0.16	0.97	121.84	0.00*
ERY	0.04	0.01	0.91	26.89	0.00*	0.08	0.03	0.89	22.38	0.00*
PEE	0.67	0.22	0.99	255.75	0.00*	0.45	0.15	0.99	271.84	0.00*
SEE	0.41	0.14	0.98	124.93	0.00*	0.30	0.10	0.97	88.03	0.00*
TVB-N										
CON	485.97	161.99	0.99	317.25	0.00*	290.39	96.80	0.96	87.38	0.00*
ERY	49.35	16.45	0.82	11.93	0.00*	222.37	74.12	0.98	145.50	0.00*
PEE	485.44	161.81	0.97	100.90	0.00*	334.52	111.51	0.99	335.57	0.00*
SEE	83.44	27.81	0.99	213.51	0.00*	237.37	79.12	0.98	108.50	0.00*
pH										
CON	0.07	0.02	0.87	18.01	0.00*	0.05	0.02	0.96	83.67	0.00*
ERY	0.57	0.19	0.98	127.78	0.00*	0.03	0.01	0.98	132.90	0.00*
PEE	0.74	0.25	0.99	222.56	0.00*	0.01	0.00	0.95	49.20	0.00*
SEE	0.75	0.25	0.99	230.88	0.00*	0.01	0.00	0.90	24.10	0.00*
*L**										
CON	17.37	5.79	0.48	2.47	0.14^{ns}	50.44	16.81	0.74	11.51	0.00*

ERY	16.59	5.53	0.51	2.83	0.11^{ns}	130.91	43.64	0.81	11.42	0.00*
PEE	18.30	6.10	0.53	3.02	0.09^{ns}	73.48	24.49	0.80	10.66	0.00*
SEE	0.70	3.13	0.39	1.71	0.24^{ns}	49.39	16.46	0.79	10.04	0.00*
*a**										
CON	46.19	15.40	0.95	2.47	0.00*	21.28	7.09	0.84	19.83	0.00*
ERY	30.46	10.15	0.91	25.95	0.00*	24.45	8.15	0.94	39.14	0.00*
PEE	19.80	6.60	0.87	17.29	0.00*	21.48	7.16	0.88	20.37	0.00*
SEE	6.62	2.21	0.78	9.24	0.01*	18.54	6.18	0.93	33.59	0.00*
*b**										
CON	5.34	1.78	0.52	2.84	0.11^{ns}	3.99	1.33	0.35	3.00	0.10^{ns}
ERY	1.37	0.46	0.27	0.98	0.45^{ns}	0.16	0.05	0.04	0.10	0.96^{ns}
PEE	1.53	0.51	0.18	0.58	0.64^{ns}	1.90	0.63	0.16	0.52	0.68^{ns}
SEE	9.40	0.23	0.13	0.39	0.76^{ns}	1.26	0.42	0.19	0.63	0.62^{ns}

SS sum of squares, MS mean square. Statistical significance: *p < 0.05, ^{ns}p > 0.05. (CON: control, ERY: erythorbate treatment, PEE: peel extract treatment, SEE: seed extract treatment).

4.3.5. Changes in the fatty acid composition of fish burgers

The main fatty acids in the composition of tambaqui (*Colossoma macropomum*) fish burgers in both treatments during storage periods were oleic acid (C18:1*n*-9), palmitic acid (C16:0), stearic acid (C18:0), and linoleic acid (C18:2*n*-6), and the average composition was 47% monounsaturated fatty acids (MUFAs), 42% saturated fatty acids (SFAs) and 11% polyunsaturated fatty acids (PUFAs), as shown in Table 5. Small-scale fluctuations in fatty acid composition were observed between treatments and different storage periods. Regarding the application of different antioxidants, significant differences between treatments were observed at the beginning of the treatment and after 90 days at -18 °C ($p < 0.05$), and there was no significant difference ($p > 0.05$) for the profile of the samples between treatments after 14 days at 4 °C, which indicates the loss of potential antioxidant effect for both treatments under the storage conditions tested.

Significant effects were observed regarding the addition of different antioxidants after 90 days under freezing on the fatty acid composition ($p < 0.05$), where treatment with sodium erythorbate (ERY) and avocado (*Persea americana* Mill.) by-product extracts (PEE, SEE) promoted differences during storage, mainly considering the content of arachidonic acid (C20:4*n*-6).

Table 5. The fatty acid content of raw fish burgers based on MSM of tambaqui (*Colossoma macropomum*) with different active compounds under different storage treatments.

Treatment/Storage	CON			ERY			PEE			SEE		
	Initial day	14.d (4 °C)	90.d (-18 °C)	Initial day	14.d (4 °C)	90.d (-18 °C)	Initial day	14.d (4 °C)	90.d (-18 °C)	Initial day	14.d (4 °C)	90.d (-18 °C)
Fatty acid content (% m/m in raw fish burgers)												
12:0	0.05 ± 0.00	0.13 ± 0.05	0.05 ± 0.00	0.05 ± 0.00	0.13 ± 0.03	0.05 ± 0.00	0.06 ± 0.01	0.11 ± 0.06	0.05 ± 0.00	0.05 ± 0.00	0.13 ± 0.06	0.05 ± 0.00
14:0	1.35 ± 0.01	1.35 ± 0.01	1.35 ± 0.01	1.36 ± 0.01^{BC}	1.40 ± 0.00^{A}	1.34 ± 0.01^{C}	1.36 ± 0.01	1.41 ± 0.02	1.35 ± 0.01	1.35 ± 0.00^{BC}	1.36 ± 0.02^{A}	1.34 ± 0.00^{C}
15:0	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.10 ± 0.00	0.10 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.00
16:0	28.72 ± 0.05^{a}	28.60 ± 0.21	28.56 ± 0.17	28.69 ± 0.02^{a}	27.84 ± 0.38	28.54 ± 0.05	28.28 ± 0.11^{b}	28.36 ± 0.04	28.45 ± 0.01	28.76 ± 0.02^{aA}	27.60 ± 0.82^{C}	28.58 ± 0.03^{B}
17:0	0.28 ± 0.00	0.28 ± 0.01	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.01	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.27 ± 0.01	0.28 ± 0.00
18:0	11.57 ± 0.01^{a}	11.33 ± 0.17	11.74 ± 0.23	11.57 ± 0.03^{a}	11.13 ± 0.14	11.53 ± 0.01	11.31 ± 0.02^{ba}	11.30 ± 0.03	11.38 ± 0.01	11.62 ± 0.00^{aA}	11.13 ± 0.26^{C}	11.50 ± 0.00^{B}
20:0	0.23 ± 0.00	0.25 ± 0.02	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.00	0.23 ± 0.00	0.23 ± 0.00	0.22 ± 0.00	0.23 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.24 ± 0.01
22:0	0.08 ± 0.01	0.12 ± 0.04	0.08 ± 0.01^{b}	0.06 ± 0.01	0.12 ± 0.02	0.09 ± 0.01^{a}	0.08 ± 0.03	0.07 ± 0.02	0.05 ± 0.00^{b}	0.08 ± 0.03	0.09 ± 0.02	0.07 ± 0.00^{ab}
24:0	0.05 ± 0.03	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00
Σ SFA	42.43	42.18	42.42	42.38	41.25	42.20	41.71	41.89	41.93	42.50	40.95	42.19
16:1*n*-7	4.71 ± 0.04	4.49 ± 0.15	4.73 ± 0.05	4.71 ± 0.05	4.74 ± 0.09	4.76 ± 0.06	4.78 ± 0.02	4.83 ± 0.02	4.79 ± 0.01	4.69 ± 0.04	4.58 ± 0.23	4.76 ± 0.01
17:1*n*-7 *cis*	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.00^{b}	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00^{b}	0.10 ± 0.00	0.12 ± 0.02	0.10 ± 0.00^{b}	0.10 ± 0.00	0.10 ± 0.01	0.13 ± 0.00^{a}
18:1 *trans*-9	0.13 ± 0.02	0.14 ± 0.02	0.10 ± 0.00	0.11 ± 0.01	0.17 ± 0.00	0.14 ± 0.03	0.12 ± 0.01	0.19 ± 0.01	0.11 ± 0.00	0.11 ± 0.01^{B}	0.14 ± 0.05^{A}	0.11 ± 0.00^{B}
18:1*n*-9	40.17 ± 0.10^{bc}	40.10 ± 0.36	39.81 ± 0.24	40.16 ± 0.00^{bc}	39.84 ± 0.45	40.28 ± 0.12	40.70 ± 0.05^{a}	40.22 ± 0.09	40.34 ± 0.01	39.98 ± 0.03^{c}	39.58 ± 0.63	40.06 ± 0.01
20:1*n*-9	1.77 ± 0.05	1.86 ± 0.03	1.93 ± 0.02	1.96 ± 0.03^{A}	1.76 ± 0.02^{B}	1.93 ± 0.01^{A}	1.78 ± 0.17	1.91 ± 0.03	2.00 ± 0.01	1.92 ± 0.01	1.86 ± 0.09	2.03 ± 0.05
Σ MUFA	46.88	46.67	46.66	47.04	46.61	47.20	47.47	47.25	47.33	46.79	46.25	47.08
18:2*n*-6	9.70 ± 0.03^{b}	10.14 ± 0.26	9.97 ± 0.21	9.70 ± 0.04^{b}	11.02 ± 0.88	9.65 ± 0.06	9.85 ± 0.01^{ab}	9.82 ± 0.08	9.78 ± 0.03	9.75 ± 0.01^{a}	11.74 ± 1.96	9.74 ± 0.01
18:3*n*-3	0.39 ± 0.00	0.45 ± 0.03	0.42 ± 0.03	0.39 ± 0.00	0.41 ± 0.00	0.39 ± 0.00	0.40 ± 0.00	0.41 ± 0.01	0.39 ± 0.00	0.39 ± 0.00	0.49 ± 0.09	0.40 ± 0.01
18:4*n*-3	0.09 ± 0.01^{A}	0.06 ± 0.01^{BC}	0.05 ± 0.00^{C}	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.00	0.08 ± 0.03	0.07 ± 0.01	0.07 ± 0.03
20:4*n*-6	0.41 ± 0.00	0.43 ± 0.01	0.40 ± 0.00^{b}	0.40 ± 0.00	0.56 ± 0.11	0.41 ± 0.00^{ab}	0.42 ± 0.00	0.46 ± 0.01	0.42 ± 0.00^{a}	0.41 ± 0.00^{B}	0.46 ± 0.01^{A}	0.42 ± 0.01^{aAB}
22:6*n*-3 DHA	0.07 ± 0.01	0.09 ± 0.02	0.09 ± 0.00	0.07 ± 0.01^{B}	0.10 ± 0.00^{A}	0.09 ± 0.01^{A}	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.10 ± 0.00	0.11 ± 0.02
Σ PUFA	10.66	11.16	10.92	10.62	12.15	10.6	10.82	10.86	10.73	10.71	12.81	10.72

Values are expressed as the means ± standard deviation. Different lowercase letters in each row indicate a significant difference between treatments ($p < 0.05$), and different uppercase letters in each row indicate a significant difference between storage conditions ($p < 0.05$). (CON: control, ERY: erythorbate treatment, PEE: peel extract treatment, SEE: seed extract treatment).

Effects on fatty acid composition among storage periods were also observed. The addition of seed extract (SEE) affected the lipid profile, significantly for some fatty acids (C14:0, C16:0, C18:0, C18:1 *trans*-9, and C20:4*n*-6) after 14 days at 4 °C ($p < 0.05$), favoring the abundance of MUFAs and PUFAs, which did not occur for the evaluation of samples after 90 days at -18 °C, which presented results more similar to the initial values. Other treatments also showed effects on the fatty acids composition. The addition of sodium erythorbate (ERY) favored the abundance of docosahexaenoic acid (C22:6*n*-3) for both storage periods ($p < 0.05$), while the control treatment (CON) showed a reduction in stearidonic acid (C18:4*n*-3) ($p < 0.05$). The effects of peel extract (PEE) under different storage conditions were not observed.

The responses on the profile of the lipid composition in refrigeration and freezing storage indicated that only the treatment with ethanolic extract of avocado by-product seed (SEE) showed a decrease in saturated acids and a greater abundance of unsaturated acids compared to the control treatment (CON). Previous studies have also indicated similar results for the favoring of unsaturated acids over the lipid profile with the application of natural extracts in a shelf life test of fish products as a response to the reducing effect of peroxidation through the capture of oxygen by phenolic compounds. This result is potentially favorable for the development of new clean label products, given that the ingestion of established acids has beneficial effects on human health (Nestel, 2000; Özogul et al., 2021; Nie et al., 2022).

4.3.6. Effects on changes in proximate composition during storage

The proximate composition of tambaqui (*Colossoma macropomum*) fish burger samples were analyzed to evaluate possible changes during storage. Significant changes were observed between the treatments and the storage period, as presented in Table 6. After 14 days at 4 °C, differences were observed in the fat content. The formulations with the addition of seed extract (SEE) and sodium erythorbate (ERY) indicated the highest percentage of fat among the samples with 13.02% and 12.75%, respectively ($p < 0.05$), while the lowest value was found for the formulation with peel extract (12.30%), and the fat rate was significantly lower for the control (CON) (12.13%) than for the other treatments. Similar results were observed for the end of freezing storage (-18 °C) after 90 days, where the lowest fat value was found for the control (CON) (12.79%), while the treatment with sodium erythorbate (ERY) showed a greater abundance of fat, followed by the seed extract (SEE) and peel (PEE) (13.17%, 12.90%, and 12.82%, respectively).

The addition of natural extract and synthetic antioxidants had beneficial effects on the protein content; the values after freezing storage (-18 °C to 90 days) were 19.64% (PEE), 19.14% (SEE), and 18.97% (ERY), and lower protein content was observed in the control treatment (CON) of 18.18% ($p < 0.05$).

Table 6. Proximal composition in samples of raw fish burgers based on MSM of tambaqui (*Colossoma macropomum*) with different active compounds under different storage treatments (%/100 g).

Storage days/Samples	Initial day	14 (4 °C)	90 (-18 °C)
CON			
Moisture	62.97 ± 0.86	61.55 ± 0.63	61.41 ± 0.35
Ash	2.25 ± 0.10	2.44 ± 0.04	2.47 ± 0.02
Fat	11.99 ± 0.78	12.13 ± 0.09[c]	12.79 ± 0.11[b]
Protein	17.45 ± 0.17	18.95 ± 0.40	18.18 ± 0.34[b]
Carbohydrate	5.34	4.93	5.14
Kcal	199.06	204.70	208.45
ERY			
Moisture	61.19 ± 0.34	61.64 ± 0.74	60.97 ± 0.25
Ash	2.44 ± 0.04	2.46 ± 0.06	2.43 ± 0.03
Fat	12.91 ± 0.10	12.75 ± 0.17[a]	13.17 ± 0.06[a]
Protein	18.55 ± 0.37	19.42 ± 0.16	18.97 ± 0.42[a]
Carbohydrate	4.91	3.73	4.45
Kcal	210.03	207.34	212.21
PEE			
Moisture	60.80 ± 1.76	61.78 ± 1.05	61.91 ± 1.49
Ash	2.38 ± 0.06	2.46 ± 0.06	2.44 ± 0.16
Fat	13.14 ± 0.28	12.30 ± 0.11[bc]	12.82 ± 0.09[a]
Protein	18.77 ± 0.52	18.78 ± 0.65	19.14 ± 0.60[a]
Carbohydrate	4.92	4.69	3.69
Kcal	213.00	204.55	206.70
SEE			
Moisture	62.24 ± 0.66	61.47 ± 0.34	61.35 ± 0.79
Ash	2.31 ± 0.03	2.40 ± 0.02	2.39 ± 0.04
Fat	12.25 ± 0.45	13.02 ± 0.05[a]	12.90 ± 0.19[a]
Protein	18.27 ± 0.63	19.41 ± 0.48	19.64 ± 0.05[a]
Carbohydrate	4.92	3.70	3.72
Kcal	203.04	209.62	209.57

Kcal: kilocalories in 100 g. Values are expressed as the means ± standard deviation. Different lowercase letters in each column indicate a significant difference between treatments ($p < 0.05$). (CON: control, ERY: erythorbate treatment, PEE: peel extract treatment, SEE: seed extract treatment).

Food self-degradation products alone do not reveal nutritional loss. However, storage conditions and active compounds shape the rates of compound formation from lipid oxidation, and protein denaturation can affect nutritional value. The results indicated that the addition of active compounds delayed the self-degradation of tambaqui fish burgers with a significant effect on the nutritional value of the investigated treatments. The observed trend for the increase in caloric value (Kcal) during storage is mainly related to the loss of moisture by dehydration; however, although the addition of active compounds has indicated beneficial effects for the maintenance of biochemical properties between treatments, from the changes observed for pH, TVB-N and TBARS values, a reduction in macronutrients such as fat and protein content was expected due to spoilage processes (Lekshmi et al., 2021). Therefore, the observed results may be related to the combination of response factors of the active compounds and the addition of functional ingredients to the formulation (oatmeal and cassava starch) in tambaqui fish burgers, which indicated similar effects in maintaining the proximate composition discussed in a previous study (Presenza et al., 2022).

4.4. Conclusion

The study provides a new comparative assessment of the use of natural plant-based compounds with a focus on the full use of products from the agri-food industry as active ingredients. The effects of the application of bioactive extracts reduced the rate of deterioration activity in tambaqui fish burgers during storage for 14 days at 4 °C and 90 days at -18 °C. Although the natural extracts showed less favorable results for the lipid oxidation products from the use of sodium erythorbate, the effects were significant in delaying the peroxidation process, inhibiting the formation of volatile nitrogenous bases, and favoring the lipid profile. In general, this study indicated that the application of ethanolic extracts based on avocado by-product at a concentration of 2000 ppm was directly related to the indices of antioxidant activity, maintenance of sensory quality and freshness, with a favorable effect on nutritional value, and in prolonging the shelf life of fish burgers during refrigeration and freezing storage.

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5. GENERAL CONCLUSIONS

The hypotheses addressed in this dissertation focused on the full use of products from the agri-food industry for conversion into food with potential relevance in terms of commerciality, acceptability, and sustainability.

The first specific objective was to optimize the fish burger formulation using mechanically separated meat (MSM) from tambaqui (*Colossoma macropomum*) as the main ingredient with the addition of oatmeal and cassava starch. The results presented in chapter 3 indicated that the objectives were achieved from the study of the mixture by the simplex-centroid design. The model was satisfactory for estimating effects on textural, functional, and sensory properties. Significant effects were shown to benefit yield properties, texture, and desirable attributes in terms of appearance and advantages in nutritional aspects. The results indicated that the benefits were better for the formulation in binary combination between the flours, with cost reduction and better sensorial acceptance. The use of MSM combined with oatmeal and cassava starch was satisfactory for the development of conventional products with improved functional and sensory properties.

The third specific objective was to investigate the potential feasibility of applying avocado (*Persea americana* Mill.) by-product extract to promote fish burger shelf life extension. Chapter 4 presents the result that achieved the proposed objective. The addition of ethanolic extracts from the peels and seeds of both was satisfactory to prolong the shelf life of the fish burger in refrigeration (4 °C) and freezing (-18 °C) storage treatments. The formation of lipid oxidation products showed greater inhibition in the use of sodium erythorbate; however, both natural extracts showed significant effects in delaying the deterioration of fish burgers. These results indicated that bioactive compounds from extracts of avocado by-products have antioxidant activity, maintenance of sensory quality, and freshness, with a favorable effect on the nutritional value of fish-based products during storage.

Finally, it was possible to obtain a fish burger formulation based on a native Brazilian species, with cost reduction, improvement in technological and sensorial properties, longer shelf life, and clean labels, which can be easily adapted for industrial application.